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FRONT COVER: *Leiopelma hamiltoni* (P. J. Bishop)

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A MODELLING APPROACH TO DETERMINE A TRANSLOCATION SCENARIO FOR THE ENDANGERED NEW ZEALAND FROG *LEIOPELMA HAMILTONI*

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A stage-structured population model was developed to predict which of nine hypothetical translocation scenarios was likely to produce the best outcome for the rare Hamilton's frog (*Leiopelma hamiltoni* McCulloch). Model outcome was measured in terms of population growth rate and probability of extinction. Only females were modelled. The model predicted that moving at least 20 female adult frogs was the best strategy, and moving subadult frogs alone, or no frogs at all was the worst in terms of mean growth rate of both populations combined. When the new population was considered separately, introducing subadults alone was the worst strategy in terms of mean growth rate and extinction probability. Extinction of the donor population was most likely when 40 adult females were removed, and the extinction risk was reduced when only 20 were removed. We consider the most reasonable management strategy - confirmed by the modelling and supporting qualitative data- is the translocation of 20 adult and 20 subadult female frogs (with the concurrent translocation of 40 males). This scenario provides a balance between risk of extinction in the donor population and probability of success in the translocated population.

Key words: amphibian conservation, population viability analysis, relocation

INTRODUCTION

Hamilton's frogs (*Leiopelma hamiltoni*) are likely to have once been relatively widespread in New Zealand (Worthy, 1987), but are now present only on one small island in the Cook Strait at the top of New Zealand's South Island (Stephens Island: but see Bell *et al.*, 1998; Holyoake *et al.*, 2001). The estimated population size of *L. hamiltoni* on Stephens Island at the time of writing was approximately 300 individuals, almost entirely living in a rock-tumble fragment of total area less than 300 m^2 (Newman, 1990; Brown, 1994; Thomson, 1996; this paper). Three adult frogs are known to reside in a second fragment on Stephens Island, less that 70 m from the main rock tumble (Brown, 1994; Tocher & Brown, 2004).

Clearing of vegetation in the early 20th century destroyed most of the forest cover on Stephens Island, and generally improved conditions for the tuatara (*Sphenodon punctatus*). *L. hamiltoni* is now confined to the rock-tumble fragment (frog bank) by high numbers of predatory tuatara and severe weather conditions that are pronounced in the absence of forest. Recent data suggest the population could be increasing, and may be reaching carrying capacity. Density has apparently quadrupled from the 58 per 100 m^2 reported in the 1970s (Newman, 1990), to 220 per 100 m^2 (this paper).

Management options for *L. hamiltoni* include enhancement of habitat on Stephens Island, captive breeding, and translocation of a specific cohort to a nearby predator-free island. Translocation to a nearby island with similar habitat is a priority management action for *L. hamiltoni* (Newman, 1996). Managers are faced with deciding how many frogs (and from which age groups) to remove from the only existing population in New Zealand in order to attempt the establishment of another. In particular, a combination of subadult and adult frogs (n=10 in total) collected at random from the donor population (frog bank) each year for three years was being considered as a translocation strategy prior to the modelling exercise carried out here (Mike Aviss, Department of Conservation, *pers. comm.* 2002).

Three translocations have been previously carried out with *Leiopelma* spp. In 1992, twelve *L. hamiltoni* were transferred from the frog bank on Stephens Island to a man-made habitat 70 m away (Brown, 1994). Although several frogs returned to their original site (Tocher & Brown, 2004), a new population founded by three frogs that remained at the release site, seems to be establishing. One hundred *L. pakeka* were translocated to a forest remnant (Boat Bay) on Maud Island in 1984-85 (Bell *et al.*, 2004). *L. pakeka* was subject to a second translocation in 1997 when 300 individuals were translocated to Motuara Island from Maud Island (Tocher and Pledger, unpublished data).

Two main conservation lessons have emerged from these translocations. Firstly, individual growth rates of *L. pakeka* were remarkably high following translocations to both Boat Bay (Ben Bell, Victoria University of Wellington, *pers. comm.* 2001) and

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Motuara Island (unpublished data), presumably lowering age to first reproduction. Secondly, there is evidence that subadults are more likely to remain in the vicinity of the release site, following a translocation, than adults. Adult *L. hamiltoni* have homed over 70 m from the release site to their original capture site (Tocher & Brown, 2004).

Intensive monitoring of *L. hamiltoni* on Stephens Island began in July 1997 to gather data on population demographics and in particular to determine the stability of the *L. hamiltoni* population over a six-year study. Data on *L. hamiltoni* juvenile survival rates in the wild are sparse, and indicate high mortality between hatching and one year of age. Juveniles are thought to have specialized habitat requirements, are more prone to desiccation and may prefer moister locations compared to older animals (Bell, 1978; Newman, 1990; Thomson, 1996). As such, juveniles are not under consideration for translocation.

Appropriate conservation management decisions can only be addressed by combining our knowledge of the life history and current status of *L. hamiltoni* populations with predictions from population modelling. A tailor-made model constructed to simulate the *L. hamiltoni* population at the frog bank is used to compare various hypothetical translocation scenarios, and to identify important assumptions and parameters relating to *L. hamiltoni*. This will ultimately guide managers and future fieldwork. In particular, we constructed a population model representing two *L. hamiltoni* populations: a donor population and a new population formed by translocation. We modelled both populations simultaneously to determine which of nine hypothetical translocation scenarios was likely to produce the best outcome for *L. hamiltoni* in terms of both population growth and extinction probability.

METHODS

Model Structure

A density-dependent, stage-structured model (Burgman *et al.*, 1993; Caswell, 2001) was created using all available life history data for *L. hamiltoni* at the frog bank. As is usual for populations in which the sex ratio is close to 1:1, only females were modelled. Two populations were considered simultaneously: a donor population (population 'D'; frog bank) from which frogs were removed to create a new population (population 'T'). The structure of the model was the same for the two populations, but the values for age at first reproduction (AFR) were allowed to be lower in population T.

We programmed the model in an Excel spreadsheet. There were three stages in the model: juveniles, subadults and adults. Time spent as a juvenile before becoming a subadult was estimated conservatively as 12 months, based on capture-recapture data collected from the frog bank. We used a "pre-breeding-census" model structure, which meant that the juvenile class contained those individuals that had survived their first year. The reason for this choice was to allow the annual fertility rate to be the product of the annual reproductive rate and first-year survival, thereby eliminating the need to specify these two parameters separately (Caswell, 2001). Time spent as a subadult before entering the adult stage was determined by the estimated mean and standard deviation of the age at first reproduction, using the 'variable stage duration' approach described in Caswell (2001).

Projections were made over a 30-year period, the known natural minimum life span of *L. hamiltoni* (unpublished data). For each run of the model we noted for both populations the annual growth rate, as well as whether the population went extinct during the projection period.

Age at first reproduction (AFR), and three vital rates were used as input parameters: the survival rate for subadults and adults, and the fertility rate (the product of reproductive rate and juvenile survival; Caswell, 2001). All three vital rate parameters were subject to some degree of uncertainty, which we incorporated into our runs of the model. In particular, data for fertility and AFR were of low quality. We used a range of values for each parameter (low, medium and high) that we hoped spanned realistic bounds, and allowed the results to be considered in the context of the full range of uncertainties.

TABLE 1. Range of values used to represent uncertainty in the parameters of the population model.

Current estimate of vital rate	Low	Medium	High
Fertility (reproductive rate x juvenile survival)	0.4	1.2	2.0
Subadult survival	0.57	0.73	0.85
Adult survival	0.80	0.88	0.93
CV for Environmental Stochasticity (ES)	0.0	0.1	0.2
Density at which minimum reached (N)	500	2000	5000
Range of vital rate values (R)	0.01	0.25	0.50
AFR for population D (years)	5	6	7
Reduction in AFR for population T (years)	0	1	2

All vital rates were set to be negatively density-dependent (i.e. to decrease with increasing density); for this purpose, density was defined as the total number of adults and subadults. The equation used to specify density-dependence is given in Appendix 1. The strength of the density-dependence was specified by four parameters (see Table 1): A, the vital rate value at the current population density; B, the range of values the vital rate can take (R; expressed as a single value representing the difference between the maximum and minimum value for that vital rate, relative to and centred on the current vital rate value); C, an arbitrarily large density at which the vital rate reaches its minimum value (N); D, an arbitrary amount of environmental stochasticity, representing year-to-year variation in the rate over and above that determined by changes in density (ES, expressed as a coefficient of variation).

Demographic stochasticity was incorporated by using a Poisson distribution to model the number of juveniles recruiting to the population, and a binomial distribution to model the number of subadults and adults surviving from one year to the next (Caswell, 2001).

The following steps were carried out for each 30-year run of the model: (1) We selected the values of the input parameters for a single 30-year run of the model. For each parameter, we selected this at random from one of three values (low, medium and high) that represented our uncertainty (i.e. one value from each line in Table 1). Using these we ran a deterministic version of the model for the donor population in order to obtain its stable stage distribution. This was then used as the initial stage distribution for that population. The current population was estimated to contain approximately 250 subadults and adults. The initial population was therefore chosen to have 125 females (subadults and adults). For some choices of parameter values, the stable stage distribution leads to there being insufficient subadults and/or adults for some of the translocation scenarios. When this occurred, we reselected the parameter values. (2) With the stable stage distribution entered into year 0, we then selected parameter values for each of the following 30 years. For year 1 and for each vital rate we used the value selected in Step 1, and those selected at random for ES, N and R (the density-dependent relationship) to determine the value for the vital rate at the current density; an example is shown in Fig. 1. For year 1 the density is that in year 0 (i.e. 125 females); for year 2 it is the density in year 1, and so on. (3) We then performed a translocation of a specified number of subadults and adults from population D to population T. Nine hypothetical translocation scenarios were modelled; Table 2.

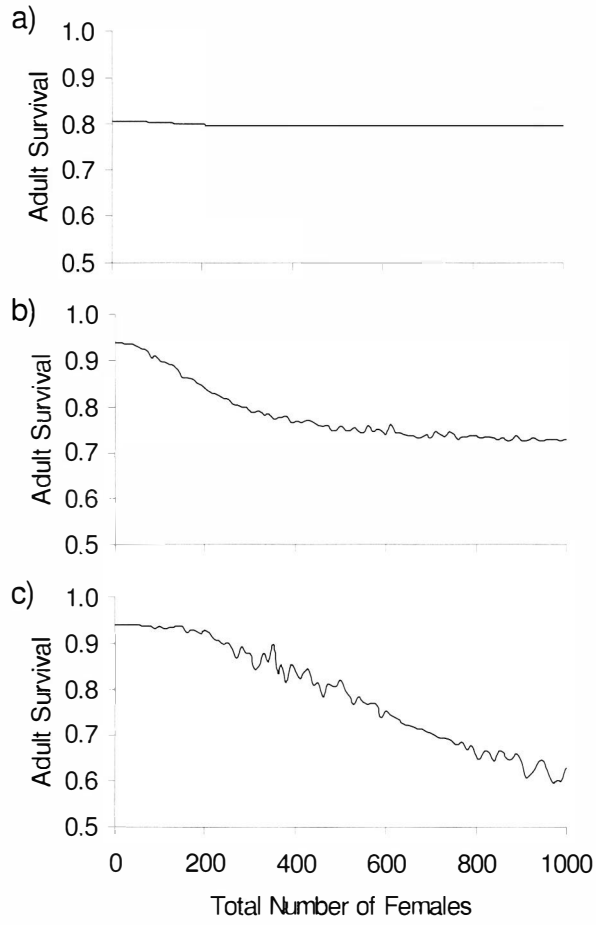

FIG. 1. Illustration of the form of density-dependence in adult survival, with the four parameters set at: a) Low values (current value=0.80, ES=0, N=500, R=0.01); b) Medium values (current value =0.88, ES = 0.1, N=2000, R=0.25; and c) High values (current value=0.93, ES=0.2, N=5000, R=0.5). See Appendix 1 for details of the equation used.

(4) We projected both populations for 30 years and recorded for each whether the population became extinct during that period as well as the annual population growth rates over the period. (5) We repeated Step 4 to obtain two replicate projections for the same translocation scenario. (6) Steps 1-5 were repeated 1000 times in order to evaluate the translocation scenarios across a wide range of possible population dynamics.

MODEL ASSUMPTIONS

Unless otherwise stated, the assumptions detailed here cannot be supported with existing data. One inherent assumption in our discussion of results is that the habitat and environment for population T is at least as good for *L. hamiltoni* as the habitat and environment of population D and that all frogs translocated to popula-

TABLE 2. Nine translocation scenarios for *L. hamiltoni* from frog bank Stephens Island to a new island site. Values represent number of subadult and adult frogs translocated.

Scenario	1	2	3	4	5	6	7	8	9
Subadults	0	0	0	20	20	20	40	40	40
Adults	0	20	40	0	20	40	0	20	40

tion T remain at the release site and become part of the population i.e. no movement away from the release site. Also, we have not allowed for population genetic effects such as inbreeding depression or phenomena that result in population dysfunction at small population sizes (e.g. Allee effects in population T). Similarly, we have assumed that any density dependence in the vital rates acts negatively, and that parameters derived from the frog bank population on Stephens Island, adequately describe population T.

We assume AFR can be lower in population T, and that changes to AFR in *L. hamiltoni* following a translocation are similar to that shown for *L. pakeka* on Maud Island (Ben Bell, Victoria University of Wellington, *pers. comm.* 2001) and Motuara Island (unpublished data). Another major assumption in our model is that the starting number of females available for translocation is 125 and that females breed annually (although we also consider 250 females and a biennial model; see Discussion). This former estimate is conservative given that mean population size from only 93 m^2 of the frog bank is estimated at 205 (Fig. 2).

Survival Rates for Adults and Subadults

Three size classes are evident in *L. hamiltoni*. Juveniles are described as frogs <16 mm SVL; subadults were defined as those 16 to 35 mm SVL inclusive, and adults >35 mm SVL (Fig. 3). We were careful to define subadults as those frogs smaller than the maximum body size for a male *L. hamiltoni* (36 to 40 mm SVL; Fig. 3). Because female *L. hamiltoni* reach a larger body size than males, this definition ensured that if a given number of subadults were selected for translocation, the sex ratio should be approximately equal (of 109 adult frogs sampled, 55 were thought to be males and 54 females).

In order to obtain estimates of mean survival rates for adults and subadults, we analysed mark-recapture data collected from the frog bank, Stephens Island, between July 1997 and July 2003 (27 sessions concentrated over autumn). All analyses were carried out using Program MARK (White & Burnham, 1999). Due to sparseness of the data, we pooled across the sexes and estimated a single survival rate for males and females. We considered four models. In all models, survival rate was constant across time, and was estimated separately for adults and subadults. Capture rate was allowed to be either time-dependent or constant, and either different for subadults and adults or not. The best-fitting model was the one in which capture rate was both time-dependent and the same for subadults and adults (AICc weight=1.000). There was some evidence of lack-of-fit for this model, which we allowed for by inflating the resulting confidence intervals using a bootstrap estimate of overdispersion (White & Burnham, 1999). The estimate of mean adult survival was 0.88 (95% confidence interval: 0.80 to 0.93), while that for subadult survival was 0.73 (95% confidence interval: 0.57 to 0.85). We used these estimates and confidence limits to specify the low, medium and high values for survival.

Fertility Rate

We decided to choose values for fertility rate so that the mean simulated population growth rate for population D (in the absence of any translocation) would match the current population growth rate. In order to estimate the latter, we used Program MARK to fit a Pradel model (White & Burnham, 1999) to the same mark-recapture data for subadults and adults. In this model, population growth rate was constant across time, and was estimated separately for adults and subadults. Following the results for the survival rate analyses, we made capture rate both time-dependent and the same for subadults and adults. We again allowed for lack-of-fit by inflating the resulting confidence intervals using a bootstrap estimate of

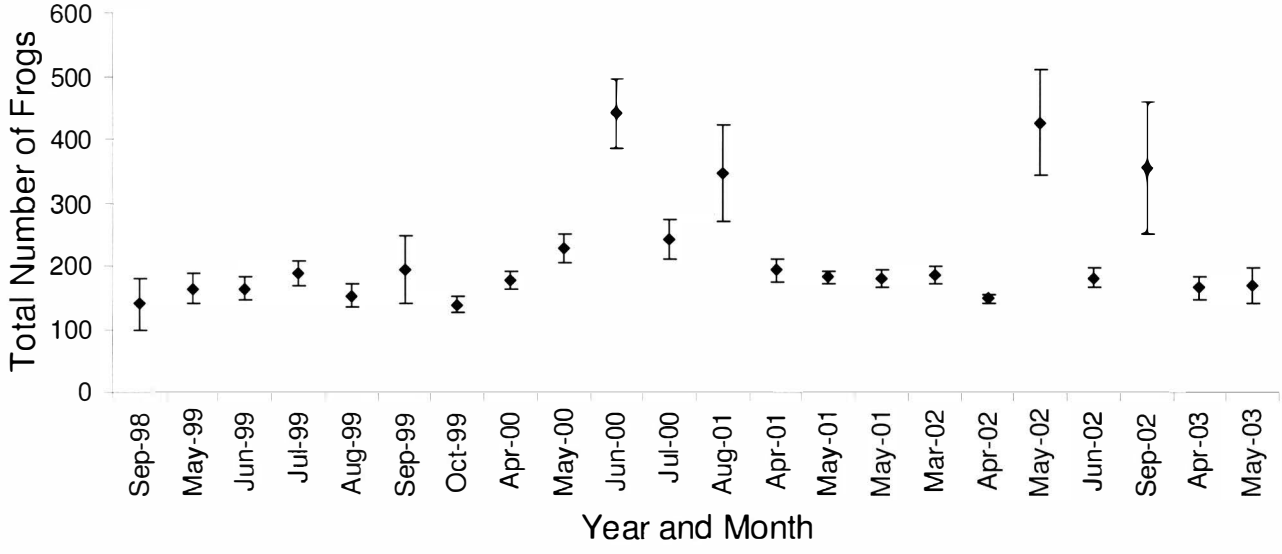

FIG. 2. Jolly-Seber population estimates (mean ± SE) for *L. hamiltoni* on Stephens Island over a 93 m^2 search area (total area of habitat for *L. hamiltoni* estimated at 300 m^2). Note: frogs were sampled twice in May 2001, early in the month, then again late in the month. Frogs < 16 mm *SVL* were not toe-clipped until session 4 therefore total population estimates were restricted to sessions 4-27 inclusive (Note: Jolly-Seber estimates cannot be calculated for the first (i.e. fourth) and last session (July 2003)).

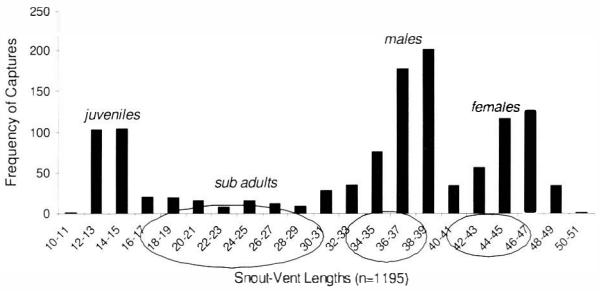

FIG. 3. Size class frequency distribution for *L. hamiltoni* on Stephens Island; n = 1195 frogs captured from 1997 to 2003.

overdispersion. The estimate of mean adult population growth rate was 1.01 (95% confidence interval: 0.85 to 1.21), while that for subadults was 0.95 (95% confidence interval: 0.88 to 1.04). The confidence intervals associated with these estimates were too wide for us to make use of them in setting low and high values for fertility rate. We therefore chose to set the values for fertility rate so that the mean simulated population growth rate for population D would equal 0.95, 1.00 and 1.05. These represent a range of plausible growth rate levels that are consistent with the data. The resulting low, medium and high values for fertility rate were 0.4, 1.2 and 2.0 respectively.

AGE AT FIRST REPRODUCTION

We assumed that individual females do not begin breeding at the same age. We specified the between-individual variation in AFR using a symmetric triangular distribution with a specified mean and an arbitrary range of four years (Fig. 4). Population T was assumed to have a mean AFR that was either zero, one or two years earlier than that for population D, again with a range of four years (Fig. 4 and Table 1). This potential reduction in AFR was considered realistic based on data collected from Motuara Island following the translocation of 300 *L. pakeka*. *L. pakeka* on Motuara Island displayed astonishing individual growth rates following translocation (unpublished data), and a similar result was noted for *L. pakeka* translocated to Boat Bay on Maud Island (Ben Bell, Victoria University of Wellington, *pers. comm.* 2001). We did not attempt to include environmental stochasticity for AFR, the same triangular distribution being used each year.

FIG. 4. Example of the triangular distributions used to represent between-individual variation in AFR. The distribution for population D has a mean AFR of six years (solid line), while that for T has a mean of five years (dashed line).

STATISTICAL ANALYSES

We summarised the results of the model runs by calculating the mean growth rate for the two populations, both separately and combined (i.e. considered as a single population). We also calculated the probability of extinction for each population. The resulting means and probabilities allow us to make comparisons amongst the different scenarios that should apply generally across a wide range of possibilities (1000 runs, each with two replicates) for the real-life dynamics of the two populations.

In order to calculate 95% confidence limits for these means and probabilities, we performed factorial ANOVAs on each measure of interest. The factors in each of these analyses were Run (1000 runs) and Translocation scenario (the nine combinations in Table 2). In each case, the ANOVA model contained the main effects of these two factors plus their interaction.The confidence intervals were calculated as ± twice the standard error of the mean or proportion concerned, with the standard error being provided as part of the ANOVA output.

As a check on the generality of the patterns in the overall means and probabilities, we also performed a sensitivity analysis by re-running the analyses of variance with a slightly different model. This model included the main effect of each model-input parameter, the main effect of the factor translocation scenario, and all possible interactions between the input parameters and this factor. The P-value corresponding to each interaction was then used as an indicator of the relative size of that interaction, and therefore of the influence of the corresponding input parameter on the comparisons amongst the translocation scenarios (actual sizes of P-values are not relevant in analysing the results of simulation studies, as they can always be "made small" simply by increasing the number of runs of the model).

RESULTS

By considering mean population growth rate over a 30-year period of population D and T combined, the model predicted the best strategies for *L. hamiltoni* involved the translocation of 40 adult (AD) frogs or a combination of 20 AD with 40 subadults (SA; Table 3).

TABLE 3. Mean population growth rate over a 30-year period for the two populations D and T combined, for each of the nine translocation strategies. Each mean has a 95% confidence interval of ± 0.002.

		Adults		
		0	20	40
Subadults	0	0.992	1.019	1.022
	20	1.013	1.021	1.020
	40	1.018	1.022	1.020

TABLE 4. Mean population growth rate over a 30-year period for the two populations considered separately, for each of the nine translocation strategies. Each mean has a 95% confidence interval of ±0.004 (population D) and ±0.006 (population T). Also shown is the predicted mean size of population T at the end of the 30-year projection period (to the nearest integer).

		Mean population growth rate						Final population size		
		population D Adults			population T Adults			population T Adults		
		0	20	40	0	20	40	0	20	40
Subadults	0	0.992	0.992	0.983		1.066	1.057		136	211
	20	0.996	0.995	0.985	0.994	1.044	1.040	17	146	195
	40	1.002	0.998	0.989	1.007	1.035	1.030	49	168	194

The 95% confidence intervals associated with values in Table 3 indicate that there is little difference in predicted growth rate between combinations involving at least 20 AD frogs. Mean combined population growth rate decreased only when 20 SA frogs alone were used for translocation, and the worst strategy was not removing any frogs at all (Table 3). It is important to note that the comparisons between these means are more robust to model-misspecification than the values themselves. The latter should not be taken as predictions of population growth rates expected for *L. hamiltoni* should each translocation scenario be carried out, as the underlying models are necessarily only approximations to reality.

When the results for the two populations were separated, scenarios involving the removal of 40 SA with 0 and 20 AD yielded the highest predicted population D growth rates (Table 4). A general trend was apparent; when scenarios which removed the same quantity of frogs were compared (across diagonals) population D growth rate decreased as more adult frogs were removed for translocation (Table 4).

For population T, modelling results were greatly dependent on the quantity of frogs translocated (i.e. the size of the founder population) and it is therefore appropriate to consider growth rates in tandem with total population size at the end of the 30-year projection period. As an example, although population T founded by 20 AD had a higher predicted population growth rate than population T founded by 40 AD, at the end of the 30-year projection period there were more frogs in population T under the latter scenario because the founder population was larger (Table 4). With this in mind, the highest growth rates were achieved in population T with the combinations that involved 20 and 40 AD (i.e. no subadults translocated to population T), and introducing subadults alone was the worst strategy (20 or 40 SA; Table 4).

Extinction probability of both populations combined was highest under the scenario involving no frogs (Table 5), a result which complements the population growth rate results (Table 3). However, when the 95% confidence intervals were considered all nine scenarios yielded statistically similar extinction probabilities (Table 5).

For populations considered separately, the best strategy for population D, in terms of extinction probability was to remove no adult frogs (Table 6). However, for population T, the more adult frogs introduced the better. The introduction of 40 AD frogs (with any combination of subadults) resulted in the lowest population T extinction probability (Table 6), and combinations involving at least 20 adults produced similar results. Of interest, the addition of 20 or 40 subadults to these 40 AD frogs resulted in no significant lowering of extinction probability. In concurrence with mean population growth results for population T, the worst scenarios in terms of extinction probability involved the introduction of either 20 or 40 SA alone (Table 6).

Sensitivity analyses indicated that population D was far less sensitive to variation in the input parameters than population T. For population T, the largest interaction involved current value for subadult survival, due in

TABLE 5. Proportion of runs that lead to extinction over a 30-year period for the two populations combined, for each of the nine translocation strategies. Each mean has a 95% confidence interval of ±0.002.

		Adults		
		0	20	40
Subadults	0	0.007	0.005	0.004
	20	0.006	0.004	0.006
	40	0.004	0.004	0.006

TABLE 6. Proportion of runs that lead to extinction over a 30-year period for the two populations separately, for each of the nine translocation strategies. Each mean has a 95% confidence interval of ±0.004 (population D) and ±0.006 (population T).

		Population D Adults			Population T Adults		
		0	20	40	0	20	40
Subadults	0	0.007	0.010	0.019		0.010	0.005
	20	0.006	0.010	0.021	0.069	0.011	0.007
	40	0.005	0.012	0.024	0.040	0.007	0.007

TABLE 7. Mean population growth rate over a 30-year period for population T, for each of nine translocation scenarios, separately for each of the three current values used for subadult survival. Each mean has a 95% confidence interval of ±0.01.

		Current subadult survival								
		0.57 Adults			0.73 Adults			0.85 Adults		
		0	20	40	0	20	40	0	20	40
Subadults	0		1.014	1.014		1.082	1.067		1.104	1.089
	20	0.838	0.992	0.994	1.054	1.060	1.052	1.090	1.080	1.074
	40	0.893	0.990	0.984	1.052	1.047	1.042	1.076	1.068	1.063

part to our wide range of values tested (Table 7). When subadult survival was low (0.57), it was most beneficial in terms of mean population growth to translocate 20 or 40 AD frogs alone. For higher values of subadult survival (0.73 and 0.85), translocating fewer adults (20 AD) became increasingly preferable, presumably due to the higher rates of subadult survival reducing the need to have such a large founder population of adults; fewer adults in the founder population lessens density dependence on vital rates culminating in an improvement in population growth rate. Overall, however, there was no significant difference between scenarios involving 20 and 40 AD, and patterns across all subadult survival values tested match patterns observed in Table 4; namely the favoured translocation scenarios involve 20 or 40 AD with the least favoured involving 20 or 40 SA alone.

The corresponding summaries for the probability of extinction in population T were similar to those for mean growth rate. Differences between scenarios were most sensitive to current value for subadult survival (Table 8). If subadult survival was low, it was best to increase the number of frogs being translocated to at least 20 AD to minimise extinction probability, with the best scenarios involving the translocation of 40 AD (Table 8). These differences were absent for the higher current values of subadult survival; here extinction probability was very low for all combinations except for 20 SA alone.

Sensitivity analyses for all other input parameters are not presented here given that different values for subadult survival (the parameter which led to the greatest interaction effect) failed to alter conclusions as to which translocation scenario was preferable, both in terms of population growth rate and extinction probability.

DISCUSSION

To fully implement the "Native Frog (*Leiopelma* spp.) Recovery Plan" (Newman, 1996), preparations must be made for a translocation of *L. hamiltoni* to another island, free of introduced mammalian predators. An appropriate island in the vicinity of Stephens Island has already been selected (Mike Aviss, Department of Conservation, *pers. comm.* 2004). To aid in preparation for a translocation we have used a tailored, species-specific simulation model, providing the degree of model complexity that is supported with available data, to choose an optimal translocation strategy for *L. hamiltoni*. The strength of our approach is that we have evaluated translocation scenarios across a wide range of possible population dynamics, and have considered risk in terms of both population growth rate and extinction.

From a choice of nine hypothetical translocation scenarios (including the "no translocation at all" option) we believe the best strategy for *L. hamiltoni* is to translocate 20 adult female frogs to a new population (with 20 adult males). Supplementing these adult frogs with 20 subadult females (with 20 subadult males) seems reasonable given that (1) subadults may be more likely to remain at the translocation site (Tocher & Brown, 2004); (2) the removal of up to 20 subadult females does not significantly impact on the population growth and extinction probability of the donor (frog bank) population; and (3) supplementing 40 translocated adult frogs with a selection of subadults improves the total number of female frogs expected in population T after 30 years, and increases the size of the founder population which

TABLE 8. Proportion of runs that lead to extinction over a 30-year period for population T, for each of nine translocation scenarios, separately for each of the three current values used for subadult survival. Each mean has a 95% confidence interval of ± 0.01.

		Current subadult survival								
		0.57 Adults			0.73 Adults			0.85 Adults		
		0	20	40	0	20	40	0	20	40
Subadults	0		0.030	0.013		0.000	0.000		0.000	0.000
	20	0.187	0.030	0.021	0.015	0.000	0.000	0.004	0.001	0.000
	40	0.118	0.019	0.021	0.002	0.000	0.000	0.000	0.000	0.000

will lessen the "bottleneck" effect and promote a relatively more diverse genetic makeup within population T.

In practical terms, *L. hamiltoni* are not easily sexed, and targeting certain sexes and ages for translocation will be necessary to ensure 40 adults and 40 subadults of an even sex ratio are translocated. We expect a random sample of frogs within 16-35 mm SVL range (subadults) to have a sex ratio slightly biased towards males (given males have a smaller adult SVL than females). As such only frogs ≥18 mm and ≤31 mm SVL should be considered for translocation to minimise a male bias in sex ratio which is most likely to occur in frogs >30 mm. We suggest a minimum SVL of 18 mm to ensure a frog is in the subadult range (given measurement error likely to occur in SVL measurements of such small frogs; Fig. 3). Likewise for adults the 40-41 mm class is likely to contain the occasional large male, but frogs >42 mm are highly likely to be female (Bell, 1994). By assuming male and female frogs are equally catchable and have similar survival rates and longevity over our 6-year study the difference between the number of frog captures in the size range 34-39 mm (455 frogs) and 42-47 mm (300 frogs) over the course of this study gives an approximate estimate of the number of females in the 34-39 mm size class range (155 frogs). Using this rough estimation, approximately 34 % of frogs within the 34-39 mm SVL size class are expected to be females. Therefore, a random collection of frogs in this size class will be male biased, and this bias can be rectified by taking frogs from within the 42-47 mm SVL range as follows: for translocation we recommend the removal of 30 adult frogs with SVLs between ≥34 and mm ≤39 mm and 10 adult frogs ≥42 mm SVL. As well, we recommend the removal of 40 subadult frogs with SVLs ≥18 mm and ≤31 mm.

Removal of 80 frogs from the frog bank represents a removal of approximately 27% of the estimated resident frog bank population, yet our modelling suggests a low probability of the donor population going extinct. To test the robustness of our conclusions to the initial size of the donor population, we repeated the full analysis using 250 rather than 125 females (subadults plus adults). This analysis produced the same patterns as those presented here, as did a model which allowed "biennial-breeding". We modelled biennial breeding because anecdotal data from our monitoring work suggested biennial breeding may indeed occur in the frog bank population with pulses of juveniles noted every second year. The biennial model generated low population growth rates (<1) and as such we considered it inferior when compared to the annual model presented here which matched the observed population growth rate of population D (unpublished data).

Our results are reassuringly robust. Sensitivity analyses showed that the results for population T were most sensitive to the current value of subadult survival. However, the overall outcome (i.e. which translocation scenario is best) was not strongly influenced by this parameter. Mark-recapture survival estimates can be prone to downward bias caused by capture heterogeneity, and we would therefore consider the medium and high values for subadult survival as more indicative of true subadult survival than the lower value and it is these values that influenced the model outcome the least. For these values the comparisons between translocation scenarios were similar to those obtained overall.

In summary, we consider the most reasonable management strategy, confirmed by the modelling and supporting qualitative data is the translocation of 20 adult and 20 subadult female frogs (with the concurrent translocation of 40 males). This scenario provides a balance between risk of extinction in donor population and probability of success in the translocated population.

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REFERENCES

Bell, B. D. (1978). Observations on the ecology and reproduction of the New Zealand leiopelmatid frogs. *Herpetologica* **34,** 340-354.

Bell, B. D. (1994). A review of the status of New Zealand *Leiopelma* species (Anura: Leiopelmatidae), including a summary of demographic studies in Coromandel and on Maud Island. *New Zealand Journal of Zoology* **21**, 341-350.

Bell, B. D., Daugherty, C. H. & Hay, J. M. (1998). *Leiopelma pakeka*, n. sp. (Anura: Leiopelmatidae), a cryptic species of frog from Maud Island, New Zealand, and a reassessment of the conservation status of *L. hamiltoni* from Stephens Island. *Journal Royal Society New Zealand* **28,** 39-54.

Bell, B. D., Pledger, S. & Dewhurst, P. L. (2004). The fate of a population of the endemic frog *Leiopelma pakeka* (Anura: Leiopelmatidae) translocated to restored habitat on Maud Island, New Zealand. *New Zealand Journal of Zoology* **31**, 123-131.

Brown, D. (1994). Transfer of Hamilton's frog, *Leiopelma hamiltoni*, to a newly created habitat on Stephens Island, New Zealand. *New Zealand Journal of Zoology* **21**, 425-430.

Burgman, M. A., Ferson, S. & Akcakaya, H. R. (1993). *Risk assessment in conservation biology*. New York: Chapman & Hall.

Caswell, H. (2001). *Matrix population models. 2nd edition.* Sunderland, Massachusetts: Sinauer Associates.

Holyoake, A., Waldman, B. & Gemmell, N. (2001). Determining the species status of one of the world's

rarest frogs: a conservation dilemma. *Animal Conservation* **4**, 29-35.

Newman, D. G. (1990). Activity, dispersion, and population densities of Hamilton's frog (*Leiopelma hamiltoni*) on Maud and Stephens Islands, New Zealand. *Herpetologica* **46**, 319-330.

Newman, D. G. (1996). *Native Frog* (Leiopelma *spp.) Recovery Plan. Threatened Species Recovery Plan No. 18.* Wellington, New Zealand: Department of Conservation, Threatened Species Unit.

Thomson, O. (1996). *Aspects of the ecology of* Leiopelma hamiltoni *(McCulloch) on Stephens Island (Takapourewa) and the implications for conservation.* BSc. Hons. Thesis, Victoria University of Wellington, New Zealand.

Tocher, M. D. & Brown, D. (2004). *Leiopelma hamiltoni* homing. *Herpetological Review* **35,** 259-261.

Usher, M. B. (1972). Developments in the Leslie matrix model. In: *Mathematical models in ecology*, 29-60. N. R. Jeffers (Ed.). Oxford, England: Blackwell.

White, G. C. & Burnham, K. P. (1999). Program MARK: survival estimation from populations of marked animals. *Bird Study* **46**, 120-139.

Worthy, T. H. (1987). Osteology of *Leiopelma* (Amphibia, Leiopelmatidae) and descriptions of three new subfossil *Leiopelma* species. *Journal of the Royal Society of New Zealand* **17**, 201-251.

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APPENDIX 1

MODEL EQUATIONS

The calculations used to determine the number of females in each stage are summarized in the equations below. Note that these are based on the assumption of a pre-breeding census, and of a variable-stage duration for subadults (Caswell, 2001).

$n_1(t+1) \sim \text{Poisson}[F(t)n_3(t)]$ (c.f. p.455 of Caswell, 2001)

$n_2(t+1) \sim \text{Binomial}[n_1(t), S_2(t)] + \text{Binomial}[n_2(t), \{1-\gamma(t)\}S_2(t)]$

$n_3(t+1) \sim \text{Binomial}[n_2(t), \gamma(t)S_3(t)] + \text{Binomial}[n_3(t), S_3(t)]$

where:

$n_1(t)$ = number of juvenile (stage 1) females in year t

$n_2(t)$ = number of subadult (stage 2) females in year t

$n_3(t)$ = number of adult (stage 3) females in year t

$F(t)$ = fertility rate (juvenile females per adult female) for adults present in year t

$S_2(t)$ = survival rate for subadult females from year t to $t+1$

$S_3(t)$ = survival rate for adult females from year t to $t+1$

$\gamma(t)$ = probability that a juvenile that survives from year t to $t+1$ becomes mature in year $t+1$

$$= \frac{1}{M} exp\left\{-\frac{1}{2} ln\left(\frac{1}{S_2(t)}\right)\left(M - \frac{V}{M}\right)\right\},$$

where M and V are the mean and variance of time spent as a subadult (Caswell, 2001). We set $M = \bar{A} - 1$, where $\bar{A}$ is the mean value of AFR (Fig. 1). Using results for the variance of a symmetric triangular distribution with a range of 4 years, we set $V = 2/3$.

Density dependence in each of the vital rates (F, S_2 and S_3) can be modelled as follows. Suppose the rate (y) is assumed to decline with population size, from a value of y_U for a population of containing one female, to a limit of y_L for an infinite population. We model the rate between the two extremes using a linear-logistic function (Usher, 1972), with the rate in a given year being calculated as:

$$y = y_U - \frac{y_U - y_L}{1 + e^{-x}} \qquad \text{(A1)}$$

where $x = a + b\ln(n)$, for some parameters a and b, and n is the total number of females in the previous year.

We specify the values of y_U and y_L as follows. The value for their difference is calculated as:

$$y_U - y_L = Ry_0$$

where R is the specified relative range of values for y (Table 1). The value for y_U is then calculated as:

$$y_U = min\left\{(1+\alpha)\, y_0^H, \left(1+\frac{R}{2}\right) y_0\right\}$$

where α is an arbitrarily small positive number, y_0 is the current value for y, and y_0^H is the highest of the three values specified for y_0 (Table 1). This choice is motivated by wanting y_U to never exceed y_0^H by more than a specified small amount, and to otherwise be such that:

$$(y_U - y_0) = (y_0 - y_L) = (y_U - y_L)/2$$

The parameters a and b are determined by setting:

(a) $y_U = y_0$ when $n = n_0$, where n_0 is the current total number of females;

(b) $y = y_L + \delta(y_U - y_L)$ when $n = N$, where N is the total number of females for which the vital rate 'reaches' y_L, with δ being an arbitrarily small positive number.

This leads to:

$$a = \frac{ln\left(\frac{d}{1-d}\right) ln(N) + ln\left(\frac{\delta}{1-\delta}\right) ln(n_0)}{ln(N) - ln(n_0)}$$

and

$$b = -\frac{a + ln\left(\frac{\delta}{1-\delta}\right)}{ln(N)}$$

where $d = (y_U - y_0)/(y_U - y_L)$.

The function in equation A1 corresponds to a deterministic density-dependent relationship. We add environmental stochasticity to the relationship by redefining x to be a normal random variable with mean $m = a + b \log n$, and standard deviation s. The latter is specified using the coefficient of variation of x as a measure of environmental stochasticity (ES). Thus s is the absolute value of mES, where the value of ES is specified in Table 1.

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VARIATION BETWEEN POPULATIONS IN THE DIET OF THE MEDITERRANEAN LIZARD *LACERTA PERSPICILLATA*

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We examined the diet of *Lacerta perspicillata* in populations from different localities, thus providing the first quantiative data on the diet of this species. Five continental populations in Morocco located at different altitudes and an introduced insular population were analysed during April. Our results confirm that *L. perspicillata* is an insectivorous species and those found at medium altitudes with comparable ecological conditions in Morocco have a similar diet. In Taza, however, both sympatric *L. perspicillata* forms have different diets. The most varied diets were observed at high altitude and in insular populations. Local diet variability is probably more related to different ecological conditions and, consequently, changing trophic availability than to lizard body size or other morphological or behavioural constraints. Further studies, including studies on trophic availability and seasonal variation, could confirm our preliminary results on local differences in the dietary habits of this species and the potential role of insularity.

Key words: altitude, feeding ecology, insularity, Lacertidae

INTRODUCTION

L. perspicillata (Duméril & Bibron, 1839) is a small North African lacertid lizard endemic to the Western Mahgreb (Bons & Geniez, 1996) presently distributed across Morocco (Medium and High Atlas, Oulmés Plateau, Debdou Mountains and some introduced populations in the Atlantic Coast) and the north-west of Algeria (Oran and Western area of the Tellian Atlas; Mateo, 1997). At an indeterminate time, it was introduced in Menorca (Balearic Islands, Spain) constituting the only non-African stable population and one of the only two insular localities of its distribution (Perera, 2002). In Morocco, this species lives in subhumid, humid, semiarid and arid Mediterranean areas from sea level up to 2800 m altitude while in Menorca it is not present above 100 m (Bons & Geniez, 1996; Perera, 2003). *L. perspicillata* is a small, very agile, climbing species that prefers rocky areas like walls, cliffs and big stones with small crevices. Despite being a relatively common species, studies concerning its biology and, in particular its feeding ecology, are very scarce (see Richter, 1986 and Perera, 2003 for review). A recent study on an introduced insular population in Menorca (Perera & Pérez-Mellado, unpublished) shows that *L. perspicillata* is insectivorous feeding mainly on terrestrial prey like beetles, spiders, ants, and less frequently flying prey like bees. Moreover, they eat fleshy fruits when available. However, we lack quantitative data about diet from natural African populations and information is reduced to field observations describing the consumption of small insects, ants, grubs, spiders, snails (Richter, 1986; Schleich *et al.*, 1996), and fruits (Doumergue, 1901).

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The aim of our study is to provide the first quantitative data about the feeding ecology of the Moroccan lizard in its native area of distribution and to compare it with the diet of an introduced insular population. To do this, we analysed lizards from five populations occupying distinct localities within its natural range in Morocco at different altitudes (Oukaïmeden, in the upper limit of its altitudinal range, and four localities at medium altitude: Debdou, Balcon d'Ito, Taza and Caves of Chiker) and one insular introduced population in Menorca, all studied during the spring period.

MATERIAL AND METHODS

Lizards from Morocco were collected during April 2003 and 2004 in five localities (Fig. 1): Oukaïmeden is a high mountain lake in the upper limit of the altitudinal distribution of the species (2650 m. a.s.l.) with typical subalpine vegetation; Gaada of Debdou is a subhumid medium high area (1500 m. a.s.l.) with low shrub vegetation; and Balcon d'Ito (1640 m. a.s.l.), Taza (1265 m. a.s.l.) and the Caves of Chiker (1480 m. a.s.l.) - the latter 15 km away from Taza - have denser vegetation dominated by *Quercus* sp. and shrublands (Fig. 1). Insular samples were also collected during April 2003 near Ciutadella (Menorca Island, Balearic Islands, Spain; Fig. 1) in an old disused quarry with typical Mediterranean shrub vegetation located at 50 m. a.s.l.

It should be noted that this taxon is undergoing a systematic revision. Bons (1968) described three different subspecies based on body size, coloration and body pattern: *L. p. perspicillata*, *L. p. chabanaudi* and *L. p. pellegrini*. However, recent phylogeographic analyses of the species, including some of the populations comprised in this study (Taza, Debdou and Menorca), show that subspecific groupings do not match with genetically identified clades (Harris *et al.*, 2003). Moreover, two different subspecies (*L. p. chabanaudi* and *L. p.*

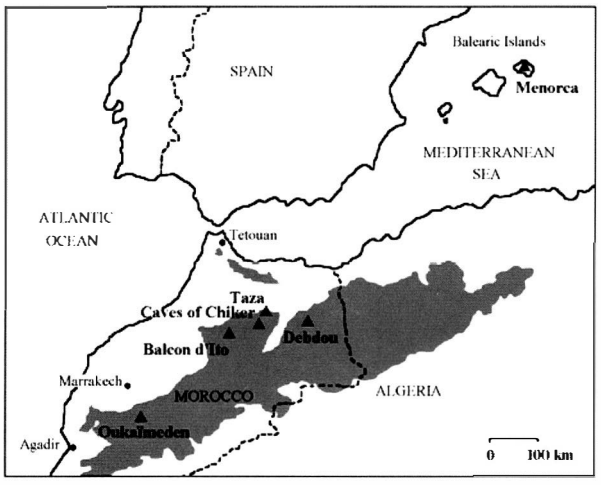

FIG. 1. Study area. Localities included in this study are indicated by triangles.

pellegrini) found syntopically in one of those localities (Taza) belong to two genetically different clades (Harris *et al.*, 2003). For this reason, we have analysed separately those two forms named hereafter as "Taza *chabanaudi*" and "Taza *pellegrini*".

Lizards were caught with a noose, sexed and the snout-vent length (SVL) and head width (HW) measured. Faecal pellets were obtained during handling, so they could be individually assigned. There are no published data about minimal body size at sexual maturity in this species. In Menorca, sexual maturity is attained during the first year at 38.5 mm and 40.0 mm of SVL in females and males respectively. Hence we used this measurement as the minimum body size for sexual maturity. We considered three different classes: 1=adult males, 2=adult females, 3=juveniles. Faeces were analysed in the laboratory through a binocular dissecting microscope. Remains were identified to the Order level, except Formicidae, which we separated from other Hymenoptera based on their non-flying aggregated behaviour. Animal prey were typologically grouped as flying (i.e. Diptera, Lepidoptera, Hymenoptera and Odonata) and terrestrial prey (all others). Intact body parts (Coleoptera elytra, Hymenoptera and Diptera wings and Homoptera and Heteroptera hemielytra) from faecal pellets were measured to the nearest 0.5 mm. Plant consumption was estimated as the percentage of vegetal matter with respect to the total amount of the pellet content.

We calculated occurrence, relative occurrence (frequency and percentage of individuals consuming a given item respectively), abundance and relative abundance (frequency and percentage of a prey item in relation to the total number of prey items respectively) for each prey type and population considered. Diet diversity was computed using the standardised version of *B* Levins's index of niche breadth (Levins, 1968), namely B_s (Hurlbert, 1978):

$$B_s = (B\text{-}1) / (n\text{-}1) = [(1/\Sigma p_j^2)\text{-}1] / (n\text{-}1)$$

where p_j^2 is the fraction of items in the diet that are of food category j, and n the number of possible food categories (Krebs, 1999). B_s ranges from 0 (100% utilization of a single food category) to 1 (equal use of all categories).

As B_s cannot be compared across groups because of the lack of associated confidence intervals, we used a delete-one Jack-knife resampling procedure (Magurran, 1988). This method entails recalculating B_s missing out each sample in turn and generating pseudovalues (VP_i), which are normally distributed. Hence, VP_i were calculated for each group and then compared using an ANOVA. Duncan or Games-Howell *post-hoc* tests for homogeneous or heterogeneous variances respectively were computed to compare differences among pairs of means (Sokal & Rohlf, 1995).

Differences between localities in diet composition and frequencies of terrestrial and flying prey were analysed with a *G*-test. We grouped low frequency items with similar attributes or behaviour to get frequencies equal or higher than 1. However, in some cases expected frequencies were lower than 5, so we performed a Monte Carlo simulation with 1000 replications and 95% confidence interval. This randomisation method allows estimation of exact significance without relying on the assumptions of the asymptotic method, like expected frequencies higher than five (Sokal & Rohlf, 1995). Multiple comparisons were corrected using sequential Bonferroni adjustment (Holm, 1979) with the program MacBonferroni (©2002 by Marley W. Watkins). We calculated Morisita's index of similarity (Krebs, 1999) to compare diet between pairwise localities. We then used the similarities matrix to perform a multidimensional scaling (MDS) to show the relationships between localities (Manly, 1986).

Lizard body measurements (SVL and HW) and mean prey sizes were log-transformed to fit the normality and homoscedasticity assumptions. Differences between localities were analysed with an ANOVA or an ANCOVA when the dependent variable covaried with another one.

The degree of association between pairs of variables was analysed with a Pearson correlation. The significance level for all tests was $\alpha = 0.05$.

RESULTS

We analysed a total of 192 pellets, all of them individually identified. We found 878 arthropod prey grouped in 21 taxa (Table 1). We detected a low occurrence of other prey items, like plant (10 pellets) or reptile remains (1 pellet).

Diet Composition

The most common prey of the Moroccan lizard were Diptera, Coleoptera, Hymenoptera, Homoptera, Araneae and larvae (all of them >5% occurrence; Table 1). However, diet composition differed between populations ($G_{42,878}$=234.7, P<0.001). The plot of the

first two dimensions of the multidimensional scaling (MDS) clearly shows these differences (Fig. 2).

The diet composition of continental samples differed ($G_{35,687}$=129.5, P<0.001). Hence, the MDS plot defines a group formed by Balcon d'Ito, the Caves of Chiker and Debdou with a similar diet based on Diptera, Coleoptera and Hymenoptera ($G_{14,284}$=16.7, P=0.357) separated from Oukaïmeden, Taza and Menorca, the former being the most distinct mainland locality. Diet composition in Oukaïmeden includes a higher consumption of Coleoptera and lower Hymenoptera consumption (Table 1). Both forms from Taza have a clearly different composition ($G_{7,249}$=15.5, P=0.038; Fig. 2) with Taza *pellegrini* having a diet more similar to the populations of Balcon d'Ito and Caves of Chiker ($G_{14,284}$=16.7, P=0.357). Thus, Taza *chabanaudi* had a higher consumption of Isoptera, Hymenoptera and larvae, whereas Taza *pellegrini* ate more Diptera and other minor items (Table 1). Finally, lizards from Menorca displayed a different diet composition than lizards from the continental populations, (G-test, $G_{7,878}$=105.2, P<0.001), with Coleoptera as the most important prey, followed by Araneae and Homoptera (Table 1).

We detected sporadic consumption of plant matter in three mainland localities: Oukaïmeden (two pellets; <10% in both cases) Debdou (one pellet, <10%) and Taza (Table 1). The latter had higher plant consumption: six *pellegrini* form lizards (mean=33.3%; min-max=10-80% of the total pellet matter) and one *chabanaudi* form lizard (30% of the total pellet matter). Remains included small stems, fragments of gramineae or seed plums, but not fruits, often occurring in a very low proportion.

Flying vs. Terrestrial Prey

The Menorcan population had a more varied diet (18 taxa) with 87% of identified items corresponding to terrestrial prey. Continental populations fed, in general, on

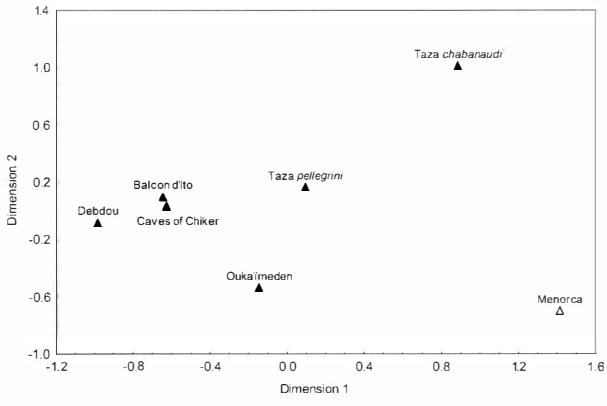

FIG. 2. Plot of *L. perspicillata* localities under study against the first two dimensions of the configuration produced by three-dimensional non-metric multidimensional scaling of a similarity matrix. Similarity matrix was performed using Morisita's index of similarity between two populations (Stress=0.001). The open symbol represents the insular population and solid symbols continental populations.

fewer prey types (10, except for Taza *chabanaudi* that fed on 14 prey types), but prey tended to be of the flying variety (Table 1). Proportions varied, however, between 69% in Debdou to 29% for the Taza *chabanaudi* form. The latter percentage was due mainly to the consumption of Isoptera by only one lizard.

Diet Diversity

Adults and juveniles (One-way ANOVA: interaction - locality × age: $F_{8,143}$=1.4, P=0.198), and males and females (interaction - locality × sex: $F_{6,144}$=2.1, P=0.057) had similar diet diversity in the localities studied. Thus, we pooled them for further analyses. We found significant differences in niche breadth between localities (ANOVA: $F_{6,184}$=5.3, P<0.001; Table 1), the Debdou population being the most specialised and Taza *chabanaudi* the most generalist. A *post-hoc* Duncan test showed three homogeneous groups: (1) Oukaïmeden, Debdou, Menorca, Taza *pellegrini* and the Caves of Chiker; (2) Oukaïmeden, Menorca, Taza *pellegrini,* Caves of Chiker and Balcon d'Ito; and (3) Taza *pellegrini*, the Caves of Chiker, Balcon d'Ito and Taza *chabanaudi*. Hence, Oukaïmeden had similar B_s to other continental and insular localities, but Taza *chabanaudi* and Balcon d'Ito, Taza *pellegrini* and the Caves of Chiker populations had similar B_s but differed from other medium altitude localities, i.e., Balcon d'Ito and Taza *chabanaudi*. Insular B_s was similar to all continental populations except Taza *chabanaudi*.

Prey Size

We lack data from juveniles for some populations, and sample sizes did not allow comparisons between age or sex classes. Hence only adult lizards were considered in this analysis. Lizard snout-vent length (SVL) and head width (HW) varied between forms (SVL: $F_{2,151}$=10.2, P<0.001; HW: ANCOVA, $F_{2,150}$=9.8, P<0.001; in both analyses Games-Howell *post-hoc* test: *chabanaudi* with other groups P<0.01, other pairwise comparisons: P>0.05) and populations (SVL: $F_{6,147}$= 6.2 P<0.001; HW: ANCOVA, $F_{6,147}$= 4.6, P<0.001; Table 2). Considering all populations together, mean prey size was positively correlated with SVL (r=0.238, P<0.01, n=118) and HW (r=0.276, P<0.01, n=118) but considering each locality separately correlation between mean prey size and lizard measurements (SVL and HW) was not significant (correlation values in all cases P>0.05). However, we used them as covariates in subsequent prey size analyses. As results did not vary using SVL or HW, only results using HW are presented. After correcting for size, individuals from Menorca fed on smaller prey than those from continental populations, except those from Debdou and Taza *pellegrini* (ANCOVA, $F_{6,110}$=2.4, P=0.032; after a Duncan *post-hoc* test, two homogeneous groups: Menorca-Debdou-Taza *pellegrini*; Debdou-Taza *chabanaudi* - Oukaïmeden-Balcon d'Ito-Caves of Chiker; Table 2). Both forms from Taza fed on similar

TABLE 1. Diet analysis of faecal samples from *L. perspicillata* from Morocco [six populations, five localities: Oukaïmeden, Debdou, Balcon d'Ito, Taza (2 distinct populations) and Caves of Chiker in April 2003 and 2004], and Menorca (one population, April 2003). %P = relative incidence, %A = relative abundance, *B*=diversity of Levins, B_s= standardized diversity of Levins.

	Oukaïmeden		Balcon d'Ito		Taza *chabanaudi*		Taza *pellegrini*		Caves of Chiker		Debdou		Menorca	
	%P	%A	%P	%A	%P	%A	%P	%A	%P	%A	%P	%A	%P	%A
Gastropoda	-	-	5.9	1.4	-	-	4	1.8	16.7	5.6	-	-	-	-
Pseudoescorpionidae	-	-	-	-	-	-	4	0.9	-	-	-	-	1.9	0.5
Aranea	9.7	1.9	11.8	2.7	31.8	5.2	32	7	16.7	5.6	9.7	1.7	44.4	13.1
Acarina	-	-	-	-	-	-	-	-	-	-	-	-	1.9	1.6
Isopoda	-	-	-	-	-	-	-	-	-	-	-	-	11.1	3.1
Chilopoda	3.2	0.6	-	-	-	-	-	-	-	-	-	-	-	-
Diplura	-	-	-	-	-	-	-	-	-	-	-	-	1.9	0.5
Orthoptera	-	-	-	-	-	-	-	-	-	-	-	-	20.4	6.3
Dictyoptera	3.2	0.6	-	-	-	-	4	0.9	-	-	-	-	7.4	2.1
Isoptera	-	-	-	-	4.5	14.1	-	-	-	-	9.7	4.6	1.9	0.5
Dermaptera	-	-	-	-	4.5	0.7	4	0.9	-	-	-	-	1.9	1.6
Homoptera	51.6	11	29.4	10.8	18.2	4.4	28	7	25	11.1	16.1	4	35.2	10.5
Heteroptera	32.3	8.4	11.8	2.7	-	-	16	3.5	16.7	5.6	-	-	3.7	1
Diptera	64.5	33.1	70.6	35.1	59.1	14.1	68	27.2	41.7	30.6	87.1	53.4	16.7	4.7
Trychoptera	-	-	-	-	-	-	-	-	-	-	3.2	0.6	-	-
Lepidoptera	-	-	-	-	-	-	4	0.9	8.3	2.8	-	-	3.7	1
Coleoptera	74.2	33.8	29.4	16.2	50	22.2	68	22.8	33.3	13.9	48.4	15.5	63	35.1
Hymenoptera	29	6.5	47.1	13.5	50	14.1	36	9.6	50	16.7	41.9	10.9	22.2	7.3
Formicidae	6.5	1.9	17.6	5.4	27.3	5.2	20	4.4	8.3	2.8	6.5	1.7	5.6	2.1
Indt. Arthrop.	9.7	1.9	29.4	6.8	13.6	2.2	12	2.6	16.7	5.6	32.3	6.3	16.7	5.2
Indt. Larvae	-	-	11.8	5.4	31.8	17.8	44	10.5	-	-	6.5	1.1	13	3.7
Indt. matter	12.9	-	11.8	-	-	-	4	-	-	-	29	-	5.6	-
Reptiles	-	-	5.9	-	-	-	-	-	-	-	-	-	-	-
Plant matter	6.5	-	-	-	4.5	-	24	-	-	-	3.2	-	-	-
%terrestrial	59.6		47.83		71.21		61.82		48.48		31.29		87.15	
%flying	40.4		52.17		28.79		38.18		51.52		68.71		12.85	
n items	10		10		10		14		10		10		18	
n pellets	31		17		22		25		12		31		54	
B	4.025		5.215		6.743		6.224		6		3.029		5.902	
B_s	0.336		0.468		0.638		0.402		0.556		0.225		0.288	

TABLE 2. Lizard body measurements (SVL and HW) and mean prey size consumed in localities under study.

	SVL(mm)		HW (mm)		Mean prey size (mm)	
	mean±SE	*n*	mean±SE	*n*	mean±SE	*n*
Oukaïmeden	58.115±1.243	26	9.502±0.291	26	3.915±0.263	23
Balcon d'Ito	58.767±3.222	15	9.703±0.546	15	3.839±0.358	12
Taza *chabanaudi*	53.050±2.749	20	8.740±0.480	20	3.686±0.351	14
Taza *pellegrini*	50.976±0.883	21	7.860±0.233	21	3.953±0.619	17
Caves of Chiker	51.375±1.920	12	7.579±0.311	12	3.826±0.868	6
Debdou	53.476±1.541	21	8.395±0.307	21	3.132±0.347	17
Menorca	47.692±0.787	39	7.813±0.183	39	2.602±0.257	29

mean size prey ($P>0.05$). We did not find a significant correlation between consumption of hard prey, such as Coleoptera and HW ($r=0.084$, $P=0.300$, $n=154$).

DISCUSSION

Previous observations on the diet of *L. perspicillata* in its native North African populations documented the consumption of snails, spiders, larvae and ants (Richter, 1986; Schleich *et al.*, 1996) suggesting that this species is insectivorous. However, Doumergue (1901) reported a lizard consuming fleshy fruits of *Rhamnus* sp. in Oran during August. On the other hand, a recent extensive study (Perera & Pérez-Mellado, unpublished) on diet composition in Menorca throughout the year confirms the insectivorous trend of this lizard in an introduced insular population and the tendency to consume fleshy fruits in the summer, as some authors previously noted (Mayol, 1985). In fact, our analysis on diet composition in continental and insular populations during the spring shows that in all cases *L. perspicillata* feeds mainly on insects such as Homoptera, Diptera, Coleoptera, Hymenoptera, larvae and other invertebrates like Araneae. However, we found differences between localities at different altitudes. Altitude variation involves important ecological changes as a consequence of differences in temperature, rainfall and other precipitations, windspeed and radiation input (Barry, 1992). Those factors may potentially influence the phenology and composition of insect communities as well as species richness (Whittaker, 1952; Gaston & Williams, 1996). For lizards located at 2800 m in the high mountainous area of Oukaïmeden, this could determine a different trophic availability and, consequently, a different diet composition to lizards found in medium altitude localities. In fact, Caves of Chiker, Taza, Balcon d'Ito and Debdou are located at 1600 m and have similar Mediterranean vegetation including *Quercus* sp. and shrublands, but with different cover density and stages of cattle exploitation. All lizards in those localities, except Taza, have a similar diet composition as a result of comparable ecological conditions, and it is likely that they share similar trophic availability. However, both syntopic *L. perspicillata* forms from Taza show some interesting differences in the abundance of consumed prey. *Chabanaudi* lizards ate a wider variety of prey, a higher proportion of which were terrestrial, whereas the diet of the *pellegrini* form varied less but had a similar proportion of both terrestrial and flying prey and, interestingly, a high consumption of plant matter. Nevertheless, mean prey size was the same in both forms. Resource partitioning between lizards can be a strategy to minimize resource competition, leading to differences in habitat use or prey utilization (Schoener, 1977; Losos, 1994). In our case, in Taza both *L. perspicillata* forms live in strict syntopy (Harris *et al.*, 2003) and recent phylogenetic analyses show that those two forms are genetically distinct (Harris *et al.*, 2003). Thus, differences in the diet could then be the result of a competitive interaction between them. Seasonal and trophic availability studies would be interesting to evaluate the real importance of this competition on food resource partitioning and its effect on diet.

The insular population from Menorca has the most distinct diet. This population consumes the highest variety of prey types, most of them terrestrial, in contrast with the Moroccan populations where flying prey are, in general, more common. Prey types consumed depend, among other factors, on the predator foraging strategy (Schoener, 1971; Pianka, 1973; Stephens & Krebs, 1986). The comparatively higher consumption of terrestrial groups, like Araneae, Coleoptera, Isopoda and Orthoptera by the insular population may suggest an active foraging strategy, while continental lizards may be closer to 'sit and wait' foragers. However, these results could be due to different trophic availability rather than a different strategy (Arnold, 1987) or just a consequence of different sample sizes among populations (sample size in Menorca was almost twice that found among some mainland populations). Current results do not allow the determination of which factors are the most influential in this species. Thus, more studies including trophic availability analyses have to be undertaken to evaluate this hypothesis.

Our results show a low consumption of plant matter among the Moroccan populations, except those in Taza where plant material is, in some cases, high. However, this does not support in any case a consistent pattern of herbivory. Plant consumption and mirmecophagy are frequent in Mediterranean lacertids and commonly related to resource scarcity due to drought periods or arid environments (Eisentraut, 1950; Pianka, 1986; Pérez-

Mellado & Corti, 1993; Van Damme, 1999). Our study was carried out in April, one of the months with higher trophic availability in Mediterranean ecosystems. A recent study (Perera & Pérez-Mellado, unpublished) on *L. perspicillata* diet variation in Menorca later in the year shows consumption of fruits and ants during the summer. Recent studies suggest that frugivory can typically be considered an insular phenomenon (Van Damme, 1999; Cooper & Vitt, 2002; Olesen & Valido, 2003; Herrel *et al.*, 2004). Interestingly, Doumergue (1901) documented consumption of fleshy fruits of *Rhamnus oleoides* by the Moroccan lizard in Oran during August indicating a potentially similar plant consumption pattern in mainland populations. Unfortunately, we lack quantitative data on summer diet confirming this field observation. Hence, further seasonal studies on the diet of the mainland populations of *L. perspicillata* could be useful to determine the existence of differences in insular and continental trophic availability as well as evaluate the possible insular effect on the diet of this species.

Diet variability of *L. perspicillata* in localities under study is probably more related to changes in ecological conditions and habitat and, consequently changing trophic availability rather than lizard body size or other morphological differences. In this way, Moroccan populations with comparable ecological conditions, except in Taza, have a similar diet, which differed from the high mountain population. Moreover, insular lizards show some peculiarities when compared to continental lizards such as different diet composition and a high proportion of terrestrial items. However, we do not know when the introduction of the Moroccan lizard in Menorca occurred (Perera, 2003), a key factor to explain potential adaptation to its new insular environment. Further studies on diet including trophic availability throughout the different seasons are needed to fully evaluate our preliminary results with regards to the potential role of insularity in this species.

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REFERENCES

Arnold, E. N. (1987). Resource partition among lacertid lizards in Southern Europe. *Journal of Zoology, London* **1**, 739-782.

Barry, R. G. (1992). Mountain climatology and past and potential future climatic changes in mountain regions-a review. *Mountain Research and Development* **12**, 71-86.

Bons, J. (1968). Révision du statut du lacertidé nord-africain *Lacerta perspicillata* Dum. et Bibr. 1839. *Bulletin de la Société de Sciences Naturelles et Physiques du Maroc* **48**, 81-92.

Bons, J. & Geniez, P. (1996). *Amphibians and Reptiles of Morocco.* Barcelona: Asociación Herpetológica Española.

Cooper, W. E. & Vitt, L. J. (2002). Distribution, extent, and evolution of plant consumption by lizards. *Journal of Zoology, London* **257**, 487-517.

Doumergue, F. (1901). *Faune herpétologique de L'Oraine.* Reprint 1972. Amsterdam: Linnaeus Press.

Eisentraut, M. (1950). Die Eidechsen der spanischen Mittelmeerinseln und ihre Rassenaufspaltung im Lichte der Evolution. *Mitteilungen aus dem Zoologischen Museum in Berlin* **26**, 1-225.

Gaston, K. J. & Williams, P. H. (1996). Spatial patterns in taxonomic diversity. In *Biodiversity: A Biology of Numbers and Difference,* 202-229. Gaston, K. J. (Ed.). Oxford: Blackwell Science.

Harris, D. J., Carretero, M. A., Perera, A., Pérez-Mellado, V. & Ferrand, N. (2003). Complex patterns of genetic diversity within *Lacerta (Teira) perspicillata*: preliminary evidence from 12S rRNA sequence data. *Amphibia-Reptilia* **24**, 386-390.

Herrel, A., Vanhooydonck, B., Joachim, R. & Irschick, D. J. (2004). Frugivory in polychrotid lizards: effects of body size. *Oecologia* **140**, 160-168.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* **6**, 65-70.

Hurlbert, S. H. (1978). The measurement of niche overlap and some relatives. *Ecology* **59**, 67-77.

Krebs, C. J. (1999). *Ecological Methodology*. Second Edition. New York: Addison-Wesley Longman.

Levins, R. (1968). *Evolution in changing environments: some theoretical explorations.* Princeton: Princeton University Press.

Losos, J. B. (1994). Integrative approaches to evolutionary ecology: *Anolis* lizards as model systems. *Annual Review of Ecology and Systematics* **25**, 467-493.

Magurran, A. E. (1988). *Ecological diversity and its measurement.* Cambridge: University Press.

Manly, B. F. J. (1986). *Multivariate statistical methods. A primer*. Second Edition. London: Chapman & Hall.

Mateo, J. A. (1997). *Lacerta perspicillata* Duméril & Bibron, 1839. In *Distribución y biogeografía de los anfibios y reptiles en España y Portugal,* 367-370. Pleguezuelos, J. M. (Ed.). Granada: Universidad de Granada.

Mayol, J. (1985). *Rèptils i amfibis de les Balears.* Palma de Mallorca: Editorial Moll.

Olesen, J. M. & Valido, A. (2003). Lizards as pollinators and seed dispersers: an island phenomenon. *Trends in Ecology and Evolution* **18**,177–181

Perera, A. (2002). *Lacerta perspicillata* Duméril & Bibron, 1839. Lagartija de Marruecos. In *Atlas y libro Rojo de los Anfibios y Reptiles de España.* (2ª impresión), 231-232. Pleguezuelos, J. M.; Márquez, R and Lizana, M (Eds.). Madrid: DGCN-AHE.

Perera, A. (2003). Lagartija de Marruecos - *Lacerta perspicillata.* In *Enciclopedia Virtual de los Vertebrados Españoles.* Carrascal, L. M., Salvador, A. (Eds.). Madrid: Museo Nacional de Ciencias Naturales. http://www.vertebradosibericos.org/

Pérez-Mellado, V. & Corti, C. (1993). Dietary adaptations and herbivory in lacertid lizards of the genus *Podarcis* from western Mediterranean islands (Reptilia: Sauria). *Bonner Zoologische Beiträge* **44**, 193-220.

Pianka, E. R. (1973). The structure of lizard communities. *Annual Review of Ecology and Systematics* **4**, 53-74.

Pianka, E. R. (1986). *Ecology and Natural History of Desert Lizards. Analyses of the Ecological Niche and Community Structure.* Princeton: Princeton University Press.

Richter, K. (1986). *Podarcis perspicillata* (Duméril und Bibron, 1839)-Brilleneidechse. In *Handbuch der Reptilien und Amphibien Europas* Band 2/II Echsen III (Podarcis), 339-407. Böhme, W. (Ed.). Wiesbaden: AULA-Verlag

Schleich, H. H., Kästle, W. & Kabisch, K. (1996). *Podarcis perspicillata* (Duméril & Bibron, 1839). In *Amphibians and Reptiles of North Africa. Biology, Systematics, Field Guide,* 441-443. Koenigstein: Koeltz Scientific Books.

Schoener, T. W. (1971). Theory of feeding strategies. *Annual Review of Ecology and Systematics* **11**, 369-404.

Sokal, R. R. & Rohlf, F. J. (1995). *Biometry.* Third Edition. New York: Freeman & Company.

Schoener, T. W. (1977). Competition and the niche. In *Biology of the Reptilia* **7,** 35-136. Gans, C and Tinkle, D. W. (Eds.). London: Academic Press.

Sokal, R. R. & Rohlf, F. J. (1995). *Biometry.* Third Edition. New York: Freeman & Company.

Stephens, D. W. & Krebs, J. R. (1986). *Foraging Theory.* Princeton: Princeton University Press.

Van Damme, R. (1999). Evolution of herbivory in lacertid lizards: effects of insularity and body size. *Journal of Herpetology* **33**, 663-674.

Whittaker, R. H. (1952). A study of summer foliage insect communities in the Great Smoky Mountains. *Ecological Monographs* **22**, 1-44.

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ANURAN TEMPORAL OCCUPANCY IN A TEMPORARY POND FROM THE ATLANTIC RAIN FOREST, SOUTH-EASTERN BRAZIL

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Temporal distribution, reproductive mode and pattern, and calling activity were recorded for an anuran community during 13 months in a temporary pond in south-eastern Brazil. Nineteen species from four families (Bufonidae, Hylidae, Leptodactylidae and Microhylidae) were recorded at the pond. Hylidae was represented by the most species, followed by the family Leptodactylidae. The reproductive diversity of the community was represented by five reproductive modes, and three reproductive patterns (prolonged, explosive and opportunistic breeders). Reproductive temporal analysis showed an anuran succession along different conditions of the pond (dry, flooded, and drying pond), probably related to specific reproductive mode and physiological tolerance to temperature and precipitation. Leptodactylid frogs were the first breeders, reproducing before the pond filled up, followed by species that lay eggs in the vegetation above water, and lastly the largest aggregation of Hylidae took place. *Stereocyclops incrassatus* (Microhylidae) was the unique explosive breeder in the community, congregating in the pond just after the first heavy rain at the beginning of the rainy season. Multiple regression analysis showed that air temperature, pond depth, and weather condition were the best predictors to explain the calling activity in anuran species. Hylid and leptodactylid frogs responded in a different way to environmental factors: in general, positive associations for hylid frogs, and negative associations for leptodactylid frogs. There were also species-specific differences in chorus attendance related to environmental factors within each family.

Key words: Anura, breeding patterns, community, reproductive pattern, succession

INTRODUCTION

Studies on anuran communities in the Atlantic Rain Forest have increased in the last 15 years (e.g. Cardoso et al., 1989; Haddad & Sazima, 1992; Rossa-Feres & Jim, 1994, 1996; Bertoluci, 1998; Eterovick & Sazima, 2000; Bertoluci & Rodrigues, 2002). However, the number of studies conducted on temporary ponds is still far below the number of studies on permanent ponds. The former have focused on reproductive aspects related to the hydroperiod of the pond, such as tadpole phenotypic plasticity (e.g. Tejedo & Reques, 1994; Blaustein *et al.*, 1999) and anuran succession (e.g. Dixon & Heyer, 1968; Heyer, 1973; Wiest, 1982). However, we know of no reports about anuran succession in temporary ponds of the Atlantic Rain Forest.

The term anuran succession used here and in community studies on temporary ponds refers to changes in species composition related to temporal resource, such as species arrival, chorus attendance and/or tadpoles phenology. This may correspond to a certain degree of temporal partitioning, so that the arrival of a new species does not necessarily result in the disappearance of another (Dixon & Heyer, 1968; Heyer, 1973; Wiest, 1982). Beyond information on species diversity, these works may provide possible association among species composition and local features, including environmental and biotic factors (Dixon & Heyer, 1968; Wiest, 1982; Gottsberger & Gruber, 2004). In turn, this may reveal community structure, which can be regulated through predictable interactions of rain, hydroperiod, predation, and competition (Semlitsch *et al.*, 1996).

Among the environmental factors affecting timing of reproduction and the length of the breeding season in tropical anurans, rainfall appears to be the most important abiotic factor (Inger, 1969; Crump, 1974; Aichinger, 1987; Wright, 1991; Donnelly & Guyer, 1994; Bevier, 1997; Gottsberger & Gruber, 2004), followed by air temperature (Bertoluci, 1998; Bertoluci & Rodrigues, 2001). Although anuran calling and breeding activities in seasonal tropical sites are intense during the rainy season, differences in the arrival of the species and reproductive phenology are associated with their reproductive mode (Gottsberger & Gruber, 2004).

In general, little effort has been directed at determining how environmental and/or biotic conditions act on community structure in temporary ponds (e.g. Dixon & Heyer, 1968; Heyer, 1973; Wiest, 1982; Gottsberger & Gruber, 2004). Herein, we describe the anuran temporal occupancy in a temporary pond, examining the possible influence of environmental factors on the breeding activity and number of individuals and species participating in chorus activity. Three major questions are addressed: (1) Does temporal occupancy show anuran succession? (2) Are calling activities of the species affected by the environmental factors air temperature, light level, pond depth, and weather condition? (3) If so, are there common pat-

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terns of responses to the environmental factors within each family and between families?

MATERIALS AND METHODS

STUDY SITE

The study site was a temporary pond with surface area of approximately 170 m^2, located in an open area at the forest edge at Palmital (22°50'48" S; 42°27'16" W), Municipality of Saquarema, State of Rio de Janeiro, south-eastern Brazil, inside the Atlantic Rain Forest domains (*sensu* Ab'Saber, 1977). Observations were made on 84 nights from July 1999 to July 2000, consisting of 411 hours of fieldwork which was conducted monthly when the pond was dry (from July to November 1999, and from March to July 2000), and fortnightly when the pond filled up (from December 1999 to February 2000). Each visit lasted a mean of five consecutive nights. In general, fieldwork started before sunset and finished around midnight, except for nights when community calling activity was recorded, when fieldwork lasted the whole night.

SURVEY OF REPRODUCTIVE ACTIVITIES

To evaluate the species reproductive period on a seasonal scale, we defined the potential reproductive period as the period when males were involved in pre-reproductive activity (calling), and defined reproductive period as the period when direct signs of reproduction (pairs in amplexus, ovulated females, clutches or tadpoles at early stages) were observed.

Reproductive temporal pattern of breeding species was defined according to the time spent in reproductive activities (permanency of chorus) in the pond: prolonged breeders (species with a continuous potential reproductive period during the rainy season, dry season or both; chorus activity continuous, with or without rain), opportunistic breeders (species with a short potential reproductive period related to a specific environmental factor, especially, rain; chorus activity in drizzling or heavy rainy nights), and explosive breeders (species with an unique potential reproductive period; chorus activity from one to seven days). Classification of species according to its reproductive mode follows Haddad & Prado (2005).

Community calling activity was measured by the number of calling species quantified during the whole night and the number of calling individuals at the time of peak activity. We counted the number of males acoustically active for each species, during the whole night, in each hour, and determined the activity peak (the time when the largest number of males present at the pond was calling).

RECORDING OF ABIOTIC FACTORS

Air temperature at 1.50 m height above ground (measured with a mercury thermometer to 0.5° C precision), pond depth (in cm) at the deepest point, and weather conditions during the night (no rain, drizzling rain, or heavy rain) were recorded during fieldwork. The categorical values for each weather condition were 1, 2 and 3, respectively. Light level (categorical measure based upon the lunar calendar – Yearly Publication of the National Observatory 1999, and 2000), and monthly rainfall (in mm; recorded at Estação Rio Mole, located approximately 10 km from the study area) were also obtained for the study period. The categorical values for light level varied from 1 (new moon) to 6 (full moon).

DATA ANALYSIS

We examined possible associations between environmental factors and community calling activity in two ways: (1) a graphical analysis of overlapping figures of monthly rainfall, mean values of air temperature and maximum pond depth (from consecutive nights), and number of calling males per month in the families Hylidae and Leptodactylidae, and (2) statistical analysis – stepwise multiple regression analyses (Zar, 1984) – to determine the relationship between environmental factors and the abundance of calling species and calling males in the whole community, in both families (Hylidae and Leptodactylidae), and between environmental factors and the abundance of calling males in each species. Only species that formed choruses (arbitrarily defined as three or more individuals calling) were analyzed in these statistical tests.

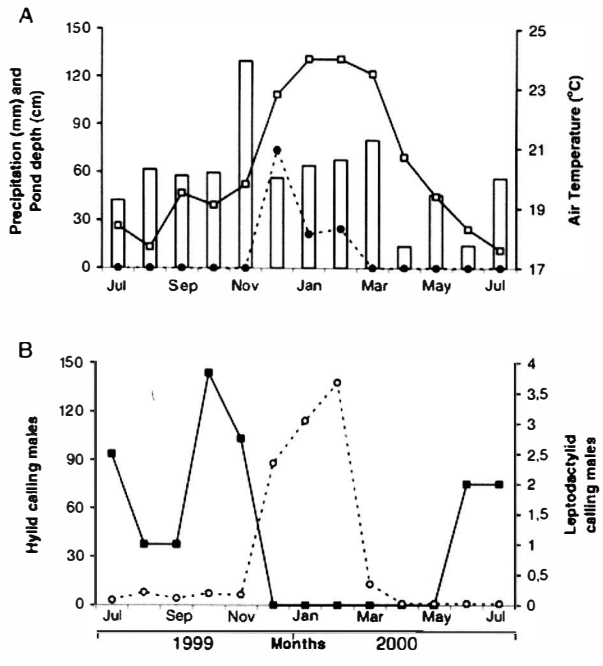

FIG. 1. A, mean air temperature (open squares), mean pond depth (filled circles) and monthly precipitation (bars) at the study site from July 1999 to July 2000. B, mean number of calling males in the families Hylidae (open circles) and Leptodactylidae (filled squares) at the study site from July 1999 to July 2000.

RESULTS

CLIMATIC ASPECTS

Climate was seasonal, with dry and rainy periods (Fig. 1). From July to November 1999, and from March to July 2000, the pond was dry; in December 1999, it was completely flooded (mean±SD pond depth = 70.4±9.8 cm, n=11; and from January to February, pond depth fluctuated according to rain (mean±SD pond depth = 23.0±7.8 cm, n=11).

SPECIES COMPOSITION

From 19 calling species belonging to four families (Bufonidae, Hylidae, Leptodactylidae and Microhylidae) recorded at the pond, 15 bred (Table 1). Hylid frogs included the highest number of species, corresponding to 68.4% of the total number, followed by leptodactylids, with 21.0%; the other families constituted together the remaining 10.6%. Reproductive diversity of the breeding community encompassed five reproductive modes: three for the breeding hylid frogs and two for the leptodactylid frogs (Table 1). The predominant reproductive mode was the most generalist (Mode 1), corresponding to 53.3% of reproductive diversity.

REPRODUCTIVE ACTIVITIES

Temporal occupancy. Potential and realized reproductive period are presented in Table 1. In general, males started to call before females arrived at the pond. Leptodactylid frogs were the pioneers, with potential and realized reproductive periods preceding the hydroperiod of the pond. Realized reproductive period of hylid frogs coincided with the period while the pond was full (although potential reproductive period from *Dendropsophus decipiens*, *Phyllomedusa burmeisteri* and *P. rohdei* began before this). *Chaunus ornatus* and *Leptodactylus spixii* did not form chorus, and for this reason, were not included on analyses.

TABLE 1. Anuran calling species of the temporary pond in Saquarema, south-eastern Brazil, from July 1999 to July 2000. Potential reproductive period (P), realized reproductive period (R), and reproductive mode *sensu* Haddad & Prado (2005): Mode 1 (eggs and exotrophic tadpoles in lentic water), Mode 4 (eggs and early larval stages in natural or constructed basins; subsequent to flooding, exotrophic tadpoles in ponds or streams), Mode 11 (foam nest floating on pond; exotrophic tadpoles in pond), Mode 24 (arboreal eggs hatching into exotrophic tadpoles that drop in lentic water), and Mode 30 (foam nest with eggs and early larval stages in subterranean constructed nests; subsequent to flooding, exotrophic tadpoles in ponds).

	1999						2000							
Species	Jul	Aug	Sep	Oc t	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Reproductive mode
BUFONIDAE														
Chaunus ornatus								P						1
HYLIDAE														
Dendropsophus anceps						R								1
D. hipunctatus						R	R	R						1
D. decipiens						R	R	R	R					24
D. elegans						R	R	R						1
D. minutus						R	R	P						1
D. seniculus							R	R						1
Hypsiboas albomarginatus								P						1
H. faber						R	P							4
Phyllomedusa burmeisteri					P	R	R	R						24
P. rohdei	P	P	P	P	P	R	R	R	R	P	P			24
Scinax argyreornatus						P	P	P						1
S. aff. *x-signatus*						R	R	R						1
Trachycephalus nigromaculatus						R		P						1
LEPTODACTYLIDAE														
Leptodactylus aff. *bokermanni*	P	R	P	R										30
L. mystacinus		P	P	R	P									30
L. spixii		P												30
Physalaemus signifer				P	R									11
MICROHYLIDAE														
Stereocyclops incrassatus						R								1

TABLE 2. Parameters of the multiple regression explaining the abundance of calling species and calling males in the families Hylidae (H) and Leptodactylidae (L), and in each chorusing species. For each predictor variable the parameters shown are the slope ± SE, *t*-test, and significance level. For multiple regression of each chorusing species, *F*-test, and the resulting *P*-value is also showed.

		Calling species			Calling males		
Family	Variables	slope±SE	t_{35}	*P*-level	slope±SE	t_{35}	*P*-level
H	Air temperature	0.31±0.09	3.33	0.002	9.28±2.63	3.53	0.001
	Light level	0.23±0.20	1.14	0.26	9.26±5.73	1.61	0.11
	Pond depth	0.05±0.01	5.50	<0.0001	0.93±0.28	3.36	0.002
	Weather condition	1.82±0.39	4.65	<0.0001	61.61±10.90	5.65	<0.001
L	Air temperature	-0.05±0.03	-1.44	0.16	-017±0.14	-1.19	0.24
	Pond depth	-0.005±0.004	-1.38	0.18	-0.02±0.01	-1.36	0.20
	Weather condition				0.64±0.58	1.19	0.24

		Calling males				
Species	Variables	slope±SE	*t*	*P*-level	*F*	*P*-value
Dendropsophus anceps	Light level	0.23±0.15	1.48	0.14	[3,36]=16.29	<0.0001
	Pond depth	0.04±0.006	6.95	<0.0001		
	Weather condition	0.58±0.29	1.95	0.06		
D. bipunctatus	Air temperature	1.86±0.62	3.00	0.005	[4,35]=16.70	<0.0001
	Light level	5.50±1.35	4.08	0.0002		
	Pond depth	0.17±0.06	2.64	0.01		
	Weather condition	14.93±2.57	5.82	<0.0001		
D. decipiens	Air temperature	0.71±0.46	1.53	0.14	[3,36]=8.32	0.0002
	Pond depth	0.07±0.05	1.42	0.16		
	Weather condition	7.16±1.79	4.00	0.0003		
D. elegans	Air temperature	1.32±0.59	2.24	0.03	[2,37]=42.54	<0.0001
	Pond depth	0.34±0.06	5.63	<0.0001		
D. minutus	Light level	-0.60±0.55	-1.09	0.28	[2,37]=4.75	0.01
	Pond depth	0.06±0.02	2.72	0.009		
D. seniculus	Air temperature	1.67±0.52	3.20	0.002	[3,36]=16.31	<0.0001
	Light level	2.46±1.42	1.74	0.09		
	Weather condition	17.35±2.69	6.45	<0.0001		
Hypsiboas albomarginatus	Air temperature	0.04±0.04	1.22	0.23	[3,36]=1.85	0.15
	Light level	0.19±0.10	1.92	0.06		
	Weather condition	0.31±0.19	1.63	0.11		
H. faber	Air temperature	0.18±0.04	4.56	<0.0001	[1,38]=20.84	<0.0001
Leptodactylus aff. *bokermanni*	Air temperature	-0.33±0.08	3.33	0.0001	[2,37]=11.84	<0.0001
	Light level	0.39±0.20	1.14	0.06		
L. mystacinus	Air temperature	-0.21±0.12	-1.79	0.08	[3,36]=11.42	<0.0001
	Light level	-0.49±0.23	-2.11	0.04		
	Pond depth	-0.04±0.01	-3.15	0.003		
Phyllomedusa burmeisteri	Air temperature	0.75±0.16	4.76	<0.0001	[3,36]=55.00	<0.0001
	Pond depth	0.10±0.02	6.14	<0.0001		
	Weather condition	2.63±0.61	4.34	0.0001		
P. rohdei	Air temperature	0.88±0.25	3.56	0.001	[3,36]=22,95	<0.0001
	Pond depth	0.09±0.02	3.59	0.0009		
	Weather condition	2.08±0.95	2.18	0.03		
Physalaemus signifer	Air temperature	-0.15±0.06	-2.50	0.02	[3,36]=4.99	0.005
	Light level	-0.29±0.16	-1.76	0.09		
	Weather condition	0.51±0.31	1.62	0.11		
Scinax argyreornatus	Pond depth	1.04±0.36	2.91	0.006	[2,37]=6.79	0.003
	Weather condition	0.02±0.008	2.60	0.01		
S. aff. *x-signatus*	Air temperature	1.06±0.49	2.14	0.04	[2,37]=6.79	0.003
	Weather condition	7.51±2.40	3.12	0.003		
Trachycephalus nigromaculatus	Air temperature	0.76±0.47	1.60	0.12	[2,37]=6.30	0.004
	Weather condition	7.52±2.30	3.26	0.002		

Among leptodactylids, *L.* aff. *bokermanni* and *L. mystacinus*, two species that build foam nests in burrows and have feeding tadpoles in ponds (after flooding), were the first to colonize the pond when it was completely dry. Potential and realized reproductive periods of *Physalaemus signifer* concentrated on drizzling rainy nights, one to two months before the pond filled completely. After leptodactylids, hylids with arboreal eggs, *D. decipiens*, *P. burmeisteri* and *P. rohdei*, initiated their realized reproductive periods, followed by *Trachycephalus nigromaculatus* and *Stereocyclops incrassatus* (Microhylidae). These last two species exhibited a punctual realized reproductive period, concentrating on the beginning of the rainy season, after heavy rain, although males of *T. nigromaculatus* also formed choruses on one night of heavy rain in February, but did not breed. A large number of clutches and tadpoles in advanced stages of *D. decipiens*, *P. burmeisteri, P. rohdei,* and tadpoles of *S. incrassatus* and *T. nigromaculatus* were found soon after the first rains of the period, indicating that the firststhree species have bred before the pond filled. A large breeding aggregation of hylid species in the pond occurred during the period while the pond was full (from December 1999 to February 2000). Hylid frogs (e.g. *D. decipiens* and *P. rohdei*) that bred late in the season (March 2000) lost their clutches.

Temporal pattern. Leptodactylid frogs were prolonged breeders during the dry season. One exception was *P. signifer,* whose choruses occurred on drizzling rainy nights (n=4), when a thin layer of water accumulated in the pond; thus, we considered it an opportunistic breeder. Most hylid frogs were prolonged breeders during the rainy season, but *D. decipiens*, *D. seniculus*, *Scinax* aff. *x-signatus* and *T. nigromaculatus* were considered opportunistic breeders. *Dendropsophus decipiens* bred on drizzling rainy nights, while *D. seniculus*, *S.* aff. *x-signatus* and *T. nigromaculatus* formed choruses just after heavy rainy nights. Only one species exhibited explosive breeding: *S. incrassatus*, mating only during the first heavy rain at the beginning of the rainy season, when the pond filled up (early December).

Abiotic factor associations. Regarding calling species and calling males of the whole community, three variables (air temperature, pond depth and weather condition) were selected by the forward step-wise model ($F_{3,36}$=24.36; P<0.001; $F_{3,36}$=15.49; P<0.001, respectively) in the following decreasing order of predictive value: pond depth, weather condition and air temperature for calling species; and air temperature, weather condition and pond depth for calling males. Concerning calling species and calling males of hylid frogs, the four variables (air temperature, light level, pond depth and weather condition) were selected by the model ($F_{4,35}$=28.43; P<0.001; $F_{4,35}$=20.75; P<0.001, respectively). For calling species of leptodactylid frogs, two variables (air temperature, and pond depth) were selected by the model ($F_{2,37}$=5.23; P<0.01), while for calling males of leptodactylid frogs, the variables selected were pond depth, weather condition, and air temperature ($F_{3,36}$=3.51; P<0.02). The predictive values of the selected variables for the regression analyses explaining the abundance of calling species and calling males in both families are in Table 2. Hylid and leptodactylid frogs responded in a different way to environmental factors. Chorus attendance in hylids was positively associated with air temperature and pond depth, while in leptodactylids it was negatively associated with the same factors. Although not all environmental factors were selected by the models – and those that were contributed in different ways – in general whenever associations occurred, they was positive for hylid species, and negative for leptodactylid species (Table 2).

Reproductive aggregations of leptodactylid frogs occurred during the dry period (dry pond), and the greatest concentration of calling males was coincident with the period of lowest air temperature (Fig. 1). In contrast, chorus attendance of hylid frogs increased with air temperature. However, the seasonal variation in the number of calling males did not follow the fluctuation of rainfall and pond depth, indicating that other factors may have contributed to the observed result.

DISCUSSION

Microhabitat diversity, niche range and niche overlap between species are non-mutually exclusive categories used to explain species diversity in a community (Inger & Colwell, 1977). Open areas offer a major horizontal distribution of calling sites for species, benefiting ground or litter species, like leptodactylid frogs, while areas with higher vegetation strata offer a vertical distribution of species related to environmental stratification, benefiting arboreal species, like hylid frogs (Cardoso *et al.*, 1989). We did not find this pattern in our study. Our data showed that although the pond was located in an open area, the family Hylidae contributed the largest number of species (68.4% versus 31.6% in the remaining families). According to Murcia (1995), species composition and the relative abundance of species can be positively or negatively affected by edge effects, depending on the taxon. The great species diversity in communities located on or near the forest edge (e.g. Blamires *et al.*, 1997; Pombal, 1997; Arzabe *et al.*, 1998; and the present study site) could be explained by the invasion of matrix-associated species not normally found in primary forest (Tocher *et al.*, 1997).

Temporal occupancy analysis showed that neither species arrival nor chorus attendance on pond were synchronized among the whole community, characterizing an anuran succession along different stages of the pond (dry and flooded). Such succession was related to the reproductive mode of the species, following the next order of appearance: leptodactylids with foam nest (*L.* aff. *bokermanni*, *L. mystacinus*, and *P. signifer)*, hylids with arboreal eggs (*D. decipiens, P. burmeisteri,* and *P. rohdei*), the explosive breeder microhylid (*S.*

incrassatus), and hylids with aquatic eggs (*D. anceps, D. bipunctatus, D. elegans, D. minutus, D. seniculus, Hypsiboas albomarginatus, H. faber, S. argyreornatus, S.* aff. *x-signatus*, and *T. nigromaculatus)*. Differences in reproductive phenology of anuran species have already been attributed to their reproductive modes (Gottsberger & Gruber, 2004), and to specific physiological characters, such as levels of tolerance to temperature and precipitation (Duellman & Trueb, 1986; Wiest, 1982).

Species that bred before the pond fills (leptodactylids with foam nest and hylid with arboreal eggs) obtained a competitive advantage of the type of nest or oviposition site, which ensures protection against desiccation, development and survival of eggs and tadpoles during a dry period (Dixon & Heyer, 1968; Duellman & Trueb, 1986). Similar patterns for foam-nesting species and leaf-breeding species were found, respectively, by Arzabe (1999) during a study conducted at a temporary pond in the Brazilian Caatinga, and by Donnelly & Guyer (1994), studying a hylid community in north-eastern Costa Rica. They observed that *Agalychnis callidryas*, as a species of the sub-family Phyllomedusinae, does not depend directly on water to breed, at least in the initial phase, as the *Phyllomedusa* species studied. Moreover, *P. burmeisteri* and *P. rohdei* liberate a great number of eggless capsules with the eggs, and fold the leaf around the eggs, allowing great moisture retention (Abrunhosa & Wogel, 2004; Wogel *et al*., 2005). The case of *D. decipiens* is similar: the only requirement for the developmental success is the synchrony between spawning and rain, since this species does not liberate eggless capsules and the clutches remain exposed, although oviposition site can be, generally, sheltered, in other words, protected by surrounded leaves (pers. obs.). The lack of rain in subsequent days from clutch deposition can be lethal to eggs, as a result of dehydration. During a study conducted at a neotropical temporary community in French-Guiana, Gottsberger & Gruber (2004) observed that breeding in *Phyllomedusa* species occurred later in the rainy season than in *Dendropsophus* species, which have arboreal eggs. The rolling of leaves around the eggs in *Phyllomedusa* species may prevent desiccation of the eggs. Sheltered oviposition sites seemed to correspond with early reproduction in *D. decipiens*. With the exception of *D. decipiens*, members of the sub-family *Phyllomedusinae* initiate reproductive aggregations before species of the sub-family Hylinae.

A stable hydroperiod at the reproductive site in temporary ponds, especially after the first rains, is one of the factors responsible for the aggregation of species that spawn directly in water (Arzabe *et al*., 1998). We observed this in the studied community for hylid species. Some of these formed choruses only after heavy rain (*D. seniculus*, *S.* aff. *x-signatus*, and *T. nigromaculatus*); others, on drizzling rainy nights (*D. decipiens*); and the majority, when the pond resembles a permanent habitat (especially in sequentially rainy nights). Similar patterns of aggregations related to rain were observed in other communities (Wiest, 1982; Aichinger, 1987; Gascon, 1991; Arzabe *et al*., 1998; Gottsberger & Gruber, 2004).

Semlitsch *et al*. (1996) observed that the annual dynamic of a temporary pond varied among years, and just when the pond filled up, many species were at the peak of their reproductive activity. During the 13 months of study, the pond was filled during a short period (about three and a half months) in December, 1999 when the major reproductive aggregation of the community was observed, based upon the number of calling species and calling males, corroborating the results of Semlitsch *et al*. (1996).

Potential reproductive period was greater than realized reproductive period for most species. In general, males started to call before females arrived at the pond, which could be important in attracting more males to increase chorus intensity, to finally attract females. However, some species (*H. albomarginatus* and *S. argyreornatus*) did not breed in this pond, and this was consistent with the small number of individuals and calling males observed.

Although we have defined reproductive temporal pattern of breeding species, it is important to clarify that these patterns can change from site to site, and among years. So, a species classified as an opportunistic breeder may have a prolonged breeding season at another site or a different year. Selective pressures at each pond can result in different reproductive temporal patterns for the same species (Wells, 1977), just as it does with *P. signifer*. In our study, this species exhibited reproductive activity only over four nights, and it was classified as an opportunistic breeder, but other populations of *P. signifer* have shown a longer period of breeding activity (see Wogel *et al*., 2002). In general, the specific conditions to initiate realized reproductive period of each species are relatively fixed: determined by reproductive mode or physical factors, but if there are no ideal breeding conditions, some species exhibit plasticity in their reproductive mode, as observed for *Hypsiboas boans* and *H. crepitans* (Caldwell, 1992), *H. rosenbergi* (Höbel, 1999), and *Physalaemus spiniger* (Haddad & Pombal, 1998). Annual patterns of calling and breeding activities of *D. minutus, D. seniculus, H. faber*, and *L. mystacinus* (see Rossa-Feres & Jim, 1994; Bertoluci, 1998; Bertoluci & Rodrigues, 2002) in other Atlantic Rain Forest sites are similar to those in our study. According to the definitions of reproductive temporal patterns established in our study, all of these species would be classified as prolonged breeders with the exception of *D. seniculus*, whose temporal pattern in that study (after copious spring rains, see Bertoluci, 1998) suggests an opportunistic breeding pattern.

The forward stepwise multiple regressions revealed that air temperature, pond depth, and weather condition were the best predictors of calling activity in anurans. Hylid and leptodactylid frogs responded in a different

way to environmental factors. Chorus attendance in leptodactylid frogs was associated with cooler nights during the dry season, while in hylid frogs it was associated with warmer nights of the rainy season. This contrasting pattern may be due to differences in reproductive mode between hylid and leptodactylid frogs. Studies in the Atlantic Rain Forest in which environmental factors (e.g. air temperature) were correlated with numbers of calling species (Bertoluci, 1998; Bertoluci & Rodrigues, 2002) did not report differences between hylid and leptodactylid frogs.

Associations between environmental factors and calling activities indicated species-specific differences in chorus attendance. All the significant negative associations between environmental factors and calling activities were exhibited by foam-nesting species. On the other hand, hylid species revealed positive associations with environmental variables. Almost all opportunistic species showed positive associations with weather conditions, except for *P. signifer* which showed strong associations with air temperature.

Concluding, anuran succession in a temporary pond depends on the particular ecology of the species involved (Barbault, 1991), specifically on the reproductive mode and reproductive temporal pattern. Temporal partitioning decreases species interactions in an anuran succession pond community (Crump, 1982; Garcia & Narins, 2000; present study), however considering time as the sole reproductive resource responsible for species coexistence is inappropriate, since congeneric species exhibited temporal overlap yet did not hybridize, showing that other factors may contribute to the reproductive isolating mechanisms.

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REFERENCES

Ab' Saber, A. N. (1977). Os domínios morfoclimáticos na América do Sul. *Geomorfologia* **52**, 1–23.

Abrunhosa, P. A. & Wogel, H. (2004). Breeding behavior of the leaf-frog *Phyllomedusa burmeisteri* (Anura: Hylidae). *Amphibia–Reptilia* **25**, 125–135.

Aichinger, M. (1987). Annual activity patterns of anurans in a seasonal neotropical environment. *Oecologia* **71**, 583–592.

Arzabe, C. (1999). Reproductive activity patterns of anurans in two different altitudinal sites within the Brazilian caatinga. *Revista Brasileira de Zoologia* **16**, 851–864.

Arzabe, C., Carvalho, C. X. & Costa, M. A. G. (1998). Anuran assemblages in Crasto Forest ponds (Sergipe State, Brazil): comparative structure and calling activity patterns. *Herpetological Journal* **8**, 111–113.

Barbault, R. (1991). Ecological constraints and community dynamics linking community patterns to organismal ecology. The case of tropical herpetofaunas. *Acta Oecologica* **12**, 139–163.

Bertoluci, J. (1998). Annual patterns of breeding activity in Atlantic rainforest anurans. *Journal of Herpetology* **32**, 607–611.

Bertoluci, J. & Rodrigues, M. T. (2002). Seasonal patterns of breeding activity of Atlantic rainforest anurans at Boracéia, southeastern Brazil. *Amphibia–Reptilia* **23**, 161–167.

Bevier, C. R. (1997). Breeding activity and chorus tenure of two neotropical hylid frogs. *Herpetologica* **53**, 297–311.

Blaustein, L., Garb, J. E., Shebitz, D. & Nevo, E. (1999). Microclimate, developmental plasticity and community structure in artificial temporary pools. *Hydrobiologia* **392**, 187–196.

Blamires, D., Motta, J. A. O., Souza, K. G. & Bastos, R. P. (1997). Padrões de distribuição e análise de canto em uma comunidade de anuros no Brasil Central. In *Contribuição ao Conhecimento Ecológico do Cerrado*, 185–190. Leite, L. L. and Saito, C. H. (Eds.). Brasília: Universidade de Brasília.

Caldwell, J. P. (1992). Diversity of reproductive modes in anurans: facultative nest construction in gladiator frogs. In *Reproductive Biology of South American Vertebrates*, 85–97. Hamlett, W. C. (Ed.). New York: Springer–Verlag.

Cardoso, A. J., Andrade, G. V. & Haddad, C. F. B. (1989). Distribuição espacial em comunidades de anfíbios (Anura) no sudeste do Brasil. *Revista Brasileira de Biologia* **49**, 241–249.

Crump, M. L. (1974). Reproductive strategies in a tropical anuran community. *Univ. Kansas Mus. Nat. History Misc.* Publ **61**, 1–67.

Crump, M. L. (1982). Amphibian reproductive ecology on the community level. In *Herpetological Communities, Wildlife Research Report*, 21–36. Scott, N. J., Jr. (Ed.).

Dixon, J. R. & Heyer, W. R. (1968). Anuran succession in a temporary pond in Colima, Mexico. *Bulletin of the Southern California Academy of Sciences* **67**, 129–137.

Donnelly, M. A. & Guyer, C. (1994). Patterns of reproduction and habitat use in an assemblage of Neotropical hylid frogs. *Oecologia* **98**, 291–302.

Duellman, W. E. & Trueb, L. (1986). *Biology of Amphibians*. New York: McGraw–Hill.

Eterovick, P. C. & Sazima, I. (2000). Structure of an anuran community in a montane meadow in southeastern Brazil: effects of seasonality, habitat, and predation. *Amphibia-Reptilia* **21**, 439–461.

Garcia-Rutledge, E. J. & Narins, P. M. (2000). Shared acoustic resources in an old world frog community. *Herpetologica* **57**, 104–116.

Gascon, C. (1991). Population- and community-level analyses of species occurrences of Central Amazonian rainforest tadpoles. *Ecology* **72**, 1731–1746.

Gottsberger, B. & Gruber, E. (2004). Temporal partitioning of reproductive activity in a neotropical anuran community. *Journal of Tropical Ecology* **20**, 271–280.

Haddad, C. F. B. & Pombal, J. P., Jr. (1998). Redescription of *Physalaemus spiniger* (Anura: Leptodactylidae) and description of two new reproductive modes. *Journal of Herpetology* **32**, 557–565.

Haddad, C. F. B & Prado, C. P. A. (2005). Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. *BioScience* **55**, 207–17.

Haddad, C. F. B. & Sazima, I. (1992). Anfíbios anuros da Serra do Japi. In *História Natural da Serra do Japi: ecologia e preservaçao de uma área florestal no sudeste do Brasil*, 188–211. Morellato, L. P. C. (Ed.). São Paulo: Editora da Universidade de Campinas.

Heyer, W. R. (1973). Ecological interactions of frog larvae at a seasonal tropical location in Thailand. *Journal of Herpetology* **7**, 337–361.

Höbel, G. (1999). Facultative nest construction in the gladiator frog *Hyla rosenbergi* (Anura: Hylidae). *Copeia* **1999**, 797–801.

Inger, R. F. (1969). Organization o communities of frogs along small rain forest streams in Sarawak. *Journal of Animal Ecology* **38**, 123–148.

Inger, R. F. & Colwell, R. K. (1977). Organization of communities of amphibians and reptiles in Thailand. *Ecological Monographs* **47**, 229–253.

Murcia, C. (1995). Edge effects in fragmented forests: implications for conservation. *Trends in Ecology and Evolution* **10**, 58–62.

Pombal, J. P., Jr. (1997). Distribuição espacial e temporal de anuros (Amphibia) em uma poça permanente na Serra de Paranapiacaba, sudeste do Brasil. *Revista Brasileira de Biologia* **57**, 583–594.

Rossa-Feres, D. C. & Jim, J. (1994). Distribuição sazonal em comunidades de anfíbios anuros na região de Botucatu, São Paulo. *Revista Brasileira de Biologia* **54**, 323–334.

Rossa-Feres, D. C. & Jim, J. (1996). Distribuição espacial em comunidades de girinos na região de Botucatu, São Paulo (Amphibia, Anura). *Revista Brasileira de Biologia* **56**, 309–316.

Semlitsch, R. D., Scott, D. E., Pechmann, J. H. K. & Gibbons, J. W. (1996). Structure and dynamics of an amphibian community. In *Long-term studies of vertebrate communities*, 217–247. Cody, M. L. and Smallwood, J. A. (Eds.). Academic Press.

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

Tocher, M. D., Gascon, C. & Zimmerman, B. L. (1997). Fragmentation effects on a Central Amazonian frog community: a ten-year study. In *Tropical Forest Remnants: Ecology, Management, and Conservation of Fragmented Communities*, 124–137. Laurance, W. F. and Bierregaard, R. O., Jr. (Eds.). Chicago Press.

Wells, K. D. (1977). The social behavior of anuran amphibians. *Animal Behaviour* **25**, 666–693.

Wiest, J. A., Jr. (1982). Anuran succession at temporary ponds in a post oak–savanna region of Texas. In *Herpetological Communities, Wildlife Research Report*, 39–47. Scott, N. J., Jr. (Ed.).

Wogel, H., Abrunhosa, P. A. & Pombal, J. P., Jr. (2002). Atividade reprodutiva de *Physalaemus signifer* (Anura, Leptodactylidae) em ambiente temporário. *Iheringia, Série Zoologia* **92**, 57–70.

Wogel, H., Abrunhosa, P. A. & Pombal, J. P., Jr. (2005). Breeding behaviour and mating success of *Phyllomedusa rohdei* (Anura, Hylidae) in south-eastern Brazil. *Journal of Natural History* **39**, 2035–2045.

Wright, S. J. (1991). Seasonal drought and the phenology on understory shrubs in a tropical moist forest. *Ecology* **72**, 1643–1657.

Zar, J. H. (1984). *Biostatistical Analysis*. London: Englewood Cliffs, Prentice-Hall.

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LONG DISTANCE MOVEMENTS BY *CAIMAN CROCODILUS YACARE*: IMPLICATIONS FOR MANAGEMENT OF THE SPECIES IN THE BRAZILIAN PANTANAL

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Movement patterns of caimans were studied over a 16-year period in two areas of the Brazilian Pantanal, one dominated by intermittent rivers and another, adjacent region of many isolated lakes. We marked caimans in 100 lakes (1986–2001) and two rivers (1987–1999). We recaptured 163 adult males, 132 adult females and 237 juveniles. In a two-year interval, hatchlings moved only within the lake area or within the river area and the maximum distance moved was 6.0 km (mean=0.5 km, SD=1.0) in the lake area, and 1.25 km (mean=0.6 km, SD=0.3) in the river area. In a period of one year, females and males larger than 40 cm snout-vent length moved similar distances in both areas (max.=9.8 km). We monitored 47 adult caimans by radio-telemetry in the river area for about a year. The size of the area used by telemetered individuals over periods of 30 to 436 days varied from two to 1649 ha. The areas used by five males in sites subjected to experimental hunting were similar to those used by five other males in areas not subjected to hunting. In periods of 1–5 years, females and males larger than 40 cm SVL moved maximum distances of 16 and 18 km, respectively. Five individuals marked as hatchlings in the lake area were recaptured as adults after intervals of 5–15 years. The extensive long-term and short-term movements by caimans mean that individual ranches should not be considered independent management units for sustained use of caimans in the Pantanal.

Key words: crocodilian, dispersal, movement, population management

INTRODUCTION

Crocodilians can move large distances in the short (Bustard & Singh, 1983; Cooper-Preston, 1992) and long term (Webb & Messel, 1978; Bustard & Choudhury, 1979). Movements may be related to reproduction (Tucker *et al.*, 1997; Coutinho *et al.*, 2000), food (Pooley & Gans, 1970; Campos, 2003), seasonal changes in water level (Schaller & Crawshaw, 1982; Ouboter & Nanhoe, 1988), or to avoid predators or pathogens (Lang, 1987). Movement patterns presumably evolved to maximize fitness of individuals under natural circumstances, but where populations are commercially exploited, movement patterns may increase the vulnerability of some segments of the population, and result in mortality patterns very different from those in populations not subject to exploitation by humans. Disturbance due to hunting may influence behaviour and movement patterns (Montague, 1983; Hutton, 1989), and hunting disturbance could lead to individuals migrating from heavily hunted areas (Campos *et al.*, 2003).

When movement patterns among and within habitats vary among demographic segments of the population, hunting strategies can be adopted that concentrate mortality on particular size or sex classes (e.g. Caughley, 1977; Joanen & Mc'Nease, 1987; Tucker *et al.*, 1997). In general, hunted populations of South American vertebrates operate as source-sink systems, with immigration from lightly hunted areas maintaining stocks in more heavily hunted regions (Bodmer, 1999).

Although short-term studies of movement are useful to determine habitat use by individuals, long-term studies are necessary to determine whether juveniles hatching in lightly hunted areas can be recruited to the size classes subject to hunting in other areas. The monitoring strategy for *Caiman crocodilus crocodilus* in Venezuela recognizes the potential for long-distance movement and quotas are based on regional estimates (Velasco *et al.*, 2003). However, in the Pantanal, monitoring of *C. c. yacare* is based on ranches as management units (Coutinho *et al.*, 1998). If adult caimans regularly move between ranches, or juveniles hatching on one ranch are regularly recruited to adult populations on neighboring ranches, ranches may be too small to function as independent management units.

Populations of *C. c. yacare* were heavily hunted in the Nhecolândia region of the Brazilian Pantanal (Mourão *et al.*, 1996), but hunting pressure was not geographically uniform. Most hunting was concentrated around intermittent rivers because these are easy to ac-

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cess, and the pools formed in the dry season have little vegetation in which caimans can hide (Coutinho & Campos, 1996). It is more difficult to access isolated lakes, and caimans in lakes are harder to locate because of emergent vegetation (Mourão *et al.*, 2000). There has been little or no hunting in our study area since 1991. Areas dominated by isolated lakes may contain populations of caimans with dynamics different from those in intermittent-river habitat. Females and clutch sizes tend to be smaller in lake areas (Campos & Magnusson, 1995), and it is generally easier to locate small caimans in lake areas (Campos *et al.*, 1995).

In this study, we investigate the movement patterns of hatchling, juvenile and adult caimans on two adjacent ranches. Campo Dora Ranch (40 000 ha) is in a region of intermittent rivers and, until the 1990s, was heavily hunted (Mourão *et al.*, 1996). Nhumirim Ranch (4300 ha) is in a region of isolated lakes, and was less intensively hunted. Both areas have been intensively studied over the last 20 years (Campos *et al.*, 1995; Coutinho & Campos, 1996; Campos, 2003). We used intensive mark-recapture and radiotelemetry studies to document movements of individuals in the short, medium and long term. Parts of Campo Dora Ranch were subject to experimental hunting in 1995 (Coutinho, 2000), and we used radio-telemetry data to determine whether the experimental hunting influenced the movement of adults that survived the hunt.

MATERIALS AND METHODS

Study Area

The study was conducted on two cattle ranches in the Nhecolândia region of the Brazilian Pantanal. Nhumirim Ranch (18°59′S and 56°39′W) is in an area characterized by isolated lakes surrounded by forest, and Campo Dora Ranch (18°55′S and 56°40′W) is characterized by flooded fields and pools associated with two intermittent rivers. The ranches are contiguous and caimans can move between the lake and river areas.

The climate in the Pantanal is classified as AW (Savanna climate) in the Köppen system. Floods are caused by local rain and/or rain in the headwaters of the rivers. The seasonal changes in the study area are related to two distinct, but related events: seasonal rains and flooding. Most rain falls between December and April (rainy season), with little or no rain between May and November, when the pools dry quickly (dry season). In the study period between 1986 and 2001, rainfall and air temperature were registered at the Nhumirim Ranch Meteorological Station (Soriano, 1997). Between January 1986 and December 1999, monthly rainfall varied from 0 to 123 mm in the dry season and 8.5 to 346.5 mm in the rainy season. Mean air temperature was 22.4°C (SD=1.8) in the cool season (May-Sept.), and 27.1°C (SD=0.8) in the warm season (Oct.-April).

Capture-recapture Study

The capture-recapture study was conducted in the lake area between 1986 and 2001, and in the river area between 1987 and 1999. Caimans were captured at night, measured and individually marked. Numbered plastic tags were fixed to raised tail scutes, and aluminium tags (National Band & Tag™ 1005-1 for hatchlings, and 1005-3 for adults) inserted in the interdigital membrane of the hind feet. Also, we cut the double and single tail scutes of each caiman in unique combinations. Recapture sessions in the lake area were undertaken monthly between 1986 and 1988, bimonthly in 1989 and once per year in 1990, 1992, 1994, 1997, 1998, 1999, and 2001. In the river area, captures of the caimans were done twice yearly from 1987 to 1994, and monthly from 1995 to 1999. Hatchlings were captured near nests from 1987 to 1999 in both areas. Adult caimans were captured with nooses at night, or seine nets during the day, and females were captured while guarding nests. All caimans were weighed (±10 g for hatchlings and ±0.5 kg for larger animals) and snout-vent length (SVL ±0.1 cm) to the posterior edge of the cloaca, was measured at each capture. We could not confidently determine the sex of caimans with SVL≤40 cm. For this reason, caimans with SVL≤40 cm were recorded as juveniles of undetermined sex. All caimans captured were used to estimate the size structure and sex ratio of populations in each area.

Movement of Hatchling Groups in the Lake Area

In 1996, eight clutches of hatchlings were monitored for periods of up to 11 months. In 1997, six clutches were monitored for periods up to 72 days. In 1999, five clutches were monitored for 120 days. The clutches were located and counted at night on the banks of the lakes, and the presence or absence of an adult (presumed to be the mother) was registered, but, on most occasions, hatchlings were not captured and identified individually.

Radio-tracking Study

In the radio-telemetry study, we used two models of radio-transmitter (33 made by Telonics™ and 14 made by Sirtrack™). The Telonics radio-transmitters measured 9.0 cm by 2.3 cm, weighed 80 g and had internal antennas. The Sirtrack transmitters measured 2.0 cm by 2.5 cm, weighed 50 g, and had external 47 cm antennas. All radio-transmitters were encapsulated in resin at the factory.

Most radios were placed between the last double tail crests and sewn to the scutes with nylon monofilament fishing line (Muñoz & Thorbjarnarson, 2000). However, 18 radios (4 Telonics, 14 Sirtrack) were implanted in the peritoneal cavity of caimans. Surgery to implant radios was done in the laboratory, under sterile conditions. The caimans were kept for one hour in a freezer to decrease their body temperature to around 19°C, which immobilized them, and a local anaesthetic (Xylocaine) was used in the region of the implant. All surgical procedures were done respecting ethical procedures for practices with animals recommended by Empresa

Brasileira de Pesquisa Agropecúaria (EMBRAPA) veterinarians.

Most caimans with radio-transmitters were not recaptured during the study to avoid disturbance. Caimans were recaptured at the end of the predicted battery life to retrieve the radio transmitters. Tracking was done with Telonics TR2 and TR4 receivers from an ultra-light aircraft, boat, car or on foot. Individuals were monitored weekly. The frequency range of the radio transmitters was 164–166 MHz. The maximum distance of signal reception at ground level was about 800 m.

Radio-tracking of caimans for more than seven months was undertaken only in the river area. Radios were fixed to the tails of 29 caimans and radios were implanted in 18 caimans. In November 1989, radio-transmitters were implanted in one male and three females. In January 1992, radios were fixed to the tails of five males and three females. In August 1993, radio-transmitters were fixed to the tails of three females and eight males. In May 1996, radios were fixed to the tails of 10 males and implanted in peritoneal cavities of seven females. In May 1999, radios were implanted in the peritoneal cavities of five males and two females.

The locations of caimans with radio-transmitters were plotted on a map of the study area. The area used by each caiman was described by a minimum convex polygon (Hayne, 1949), in the Systat 8.0 program. The area covered by the polygon was estimated by the number of pixels covering each area in Adobe Photoshop 4.0 images. The size of the pixels was determined from known areas of 25 km^2 and 100 km^2. Only caimans that were tracked for 30 days or more were used in the analyses.

CAIMANS RADIO-TRACKED IN AREAS WITH AND WITHOUT HUNTING

In August 1995, four blocks of 24 km^2 were delimited in the river area. The distances between blocks were around 4 km. Two blocks were not hunted (controls) and controlled harvesting was undertaken in the other blocks. In the hunted blocks, 648 adult caimans (SVL>80 cm) were killed (Coutinho, 2000). Between May 1996 and May 1997, 10 adult males (SVL≥ 90 cm) were radio-tracked. Two or three caimans were tracked in each block, for a total of five caimans in control blocks and five caimans in hunted blocks.

INDEX OF DISTANCE MOVED

The maximum distance moved by caimans monitored by radio-telemetry was calculated as the maximum straight-line distance between positions that the caiman was recorded. To maintain independence of the capture-recapture data, only the first and last captures of each individual were used. The distances moved between captures were measured on a LANDSAT satellite image of the study area, at a scale of 1:100,000.

CONDITION INDEX

The individual condition of the caimans with radio-transmitters was estimated as the residual of the linear regression between log body mass (logM – kg) and log snout-vent length (logM=10.25+2.906 × logSVL; r^2 =0.95; n=47; P<0.001).

RESULTS

CAPTURE-RECAPTURE

We marked 2576 caimans in the lake area and 3042 caimans in the river area between 1986 and 2001. In the 16 years of the study, 532 caimans (9.5%) were recaptured (237 hatchlings, 163 males and 132 females) over varying time intervals. The size structure of the populations varied between the lake and river areas (Fig. 1). The proportion of caimans with SVL≤40 cm was 72% in the lake area and 13% in the river area.

Of the 237 hatchlings, 67% remained in the lake in which they were captured for up to two years, and 33% moved to other lakes. In six reproductive seasons, 197 hatchlings less than one year old were recaptured. Of these, 186 moved distances up to 6.0 km (mean=0.5 km, SD=1.0) between lakes in the lake area. Eleven hatchlings in the river area moved <1.2 km (mean=0.6 km, SD=0.3) and remained in the river area. No individuals were recorded moving from the lake to the river area, or vice versa, during the first two years of life (Fig. 2). Although 197 hatchlings were recaptured within the first year of life, most did not move independently. Fig. 2 shows only the 34 independent movements. Animals that moved to and from the same places were not considered to have moved independently, and group movements are represented by single arrows.

In the reproductive seasons of 1996, 1997 and 1999, we monitored the movement of eight, six and five hatchling groups, respectively, that remained together with females in the lake area. These hatchlings were not captured at each survey, so we can only assume that most individuals remained in their original groups. However, it is extremely unlikely that a hatchling group

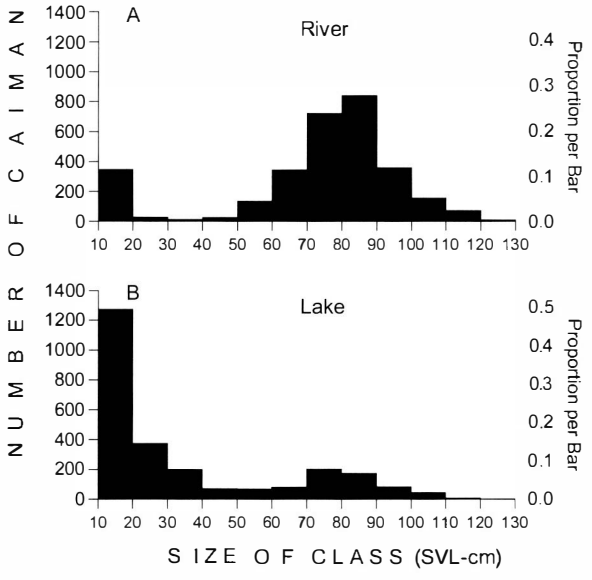

FIG 1. Sizes of caimans captured in the river area (A) and the lake area (B).

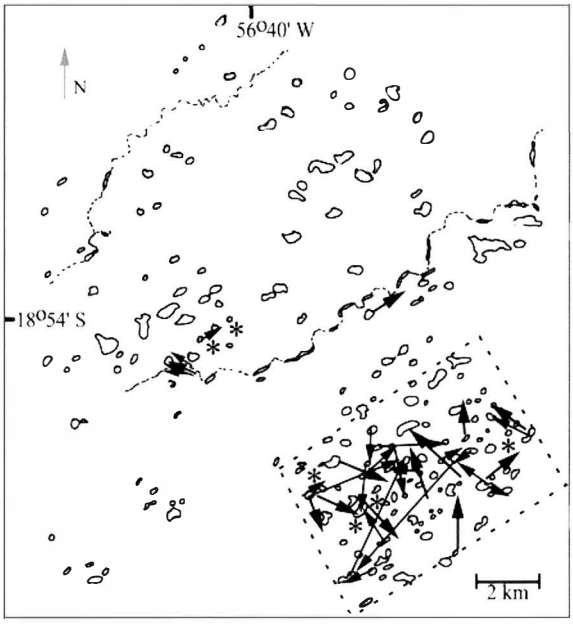

FIG 2. Movements of hatchlings (SVL≤40 cm) during intervals of up to two years, between lakes (arrows) and recaptured in the same lake (asterisks). If all hatchlings in a groups were captured and recaptured at the same places, they are represented only as a single arrow. The straight broken lines indicate the boundary between the two ranches, which corresponds to the boundary between the lake area (south of the southern limit) and the river area (north of the southern limit).

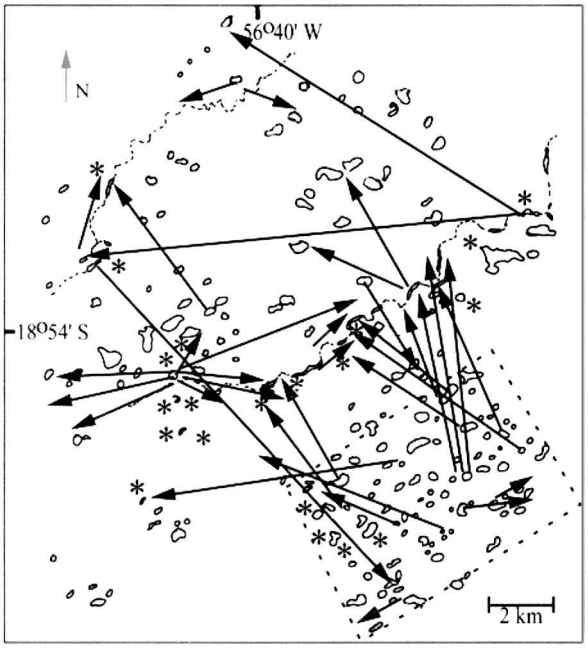

FIG 3. Movement of caimans recaptured after intervals of 1–5 years in lake and river areas. Seven caimans found dead at hunting sites are indicated with crosses. Asterisks indicate locations of hunting sites. The straight broken line indicates the boundary between the two ranches, which corresponds to the boundary between the lake area (south of the line) and the river area (north of the line).

moved and was replaced by another hatchling group of similar size in the same place. In 1996, hatchling groups remained together in the same lake for 74–330 days. In 1997, hatchling groups could be encountered for 72 days. Individuals in one of the clutches moved to another lake 500 m from where they hatched in an interval of 30 days, and the other clutches remained in the lakes were they were first recorded. In 1999, we were able to follow hatchlings for 120 days. One of the clutches moved from one lake to another 500 m away, and the others remained in the original lakes.

The maximum distances between captures recorded for males and females with SVL≥40 cm during periods up to 364 days were similar (males: max.=9.5 km, mean=1.33, SD=1.97, females: max.=9.8 km, mean=1.6 km, SD=2.05). About 30% of the variance in distance moved, D (m), by caimans could be accounted for by a multiple regression including interval between captures (I, in days), snout-vent length (SVL, in cm) and water level (L, in cm) ($D = -4868 + 1.27 \times I + 49.82 \times SVL + 22.58 \times L$; $R^2 = 0.32$, $F_{3,47} = 7.23$, $P<0.001$). SVL ($P\leq0.001$) and mean water level in the period between captures ($P=0.002$) contributed significantly to the model, but interval between captures did not ($P=0.487$).

The majority of caimans were recaptured at intervals of 1–5 years. In these periods, some animals were recaptured within the same ranch, but many moved between the river and lake areas (Fig. 3). Individuals recaptured in the same place may have undertaken more extensive movements, but this cannot be determined from capture-recapture data. Most movements were within or towards the river area. Only four of 16 individuals marked in the lake area were recaptured in the lake area. Only one of 19 individuals marked in the river area that were recaptured moved to the lake area. Most individuals that were recaptured moved to the river area ($n=12$) or remained there ($n=18$). The maximum distance between captures in <5 years was 18 km (mean=5.0 km, SD=4.3).

The mean distances moved during 1–5 years by males and females were similar and not significantly different ($t_{66}=0.318$, $P=0.751$); males moved an average distance of 2.3 km (SD=3.2) and females moved a mean of 2.1 km (SD=3.4). About 30% of the variance in distances (D, m) moved by caimans in 1–5 years could be predicted by a multiple regression including interval between captures, I (days), SVL (cm) and water level (L, cm): $D = -6756 - 2.92 \times I + 55.3 \times SVL - 73.6 \times L$ ($R^2=0.29$, $F_{3,38}=5.19$, $P=0.004$). SVL ($P=0.038$) and interval between captures ($P=0.004$) contributed significantly to the model, but the evidence for an effect of water level was equivocal ($P=0.079$).

Twenty-one caimans were recaptured after periods ≥5 years. The maximum interval between recaptures was 14 years. The relationships between distance moved (D, in km) and log SVL (cm) at first capture for the 21 caimans were described by the following equation: $D = 25.9 - 5.4 \times \log SVL$ ($R^2=0.61$, $F_{1,19}=29.5$, $P=0.001$). Although the log-linear equation described most of the data well, the area predicted to be used by large individuals (0 ha) was unrealistic (Fig. 4). Radio-telemetry

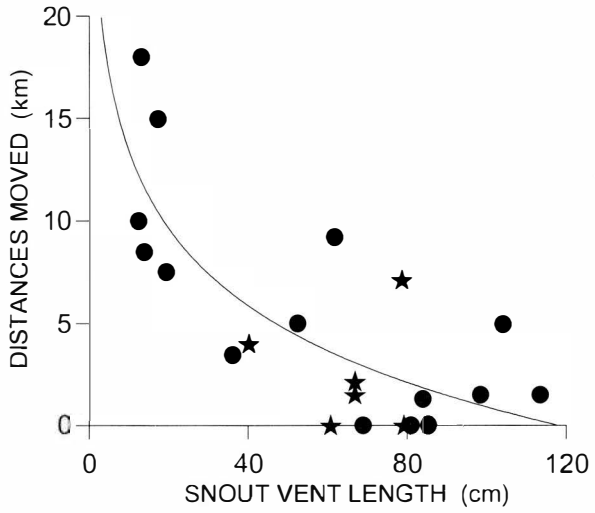

FIG. 4. Relationship between distances moved and snout-vent length at first capture for 21 male (circles) and female (stars) caimans, captured over periods of 5–15 years.

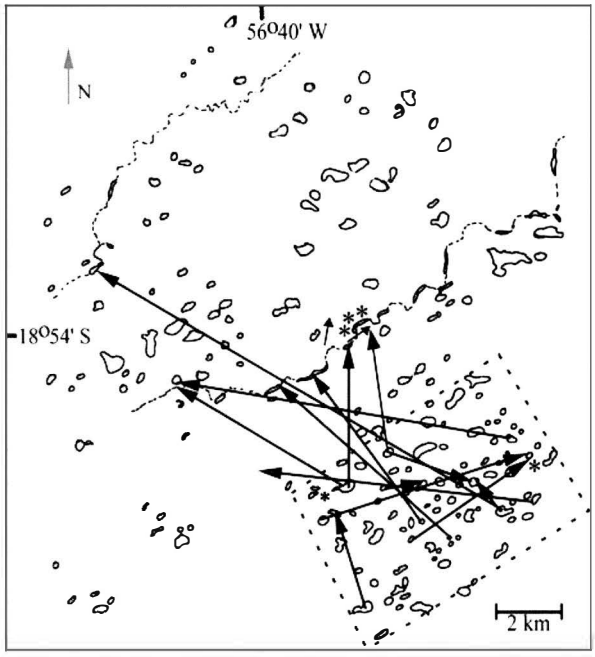

FIG. 5. Movements of 21 caimans recaptured after periods of 5–15 years. Asterisks indicate caimans that were recaptured at the site at which they were first captured. The broken line indicates the boundary between the two ranches which corresponds to the boundary between the lake area (south of the line) and the river area (north of the line).

data (see above) indicates that the mean linear distance covered by large individuals over periods in excess of one year is about 4.6 km. Based on Fig. 4, the area used by individuals approaches this value at a snout-vent length of about 52 cm, suggesting that juveniles establish fixed home ranges at about this size.

As in the medium-term movements, most of the long-term movements were from the lake area to the river area (Fig. 5). Four of the five males that were marked in the first year of life in the lake area were recaptured as adults in the river area 6–9 years later, 8–18 km from where they were marked (Table 1). One individual was recaptured in the lake area at 10 years of age, 7.5 km from where it was marked as a hatchling.

TABLE 1. Sizes of the caimans recaptured over intervals of 5 and 15 years and maximum distances between capture points in the river (R) and in lake areas (L).

Sex	Date of capture	Capture habitat	SVL (cm)	Mass (kg)	Date of recapture	Recapture habitat	SVL (cm)	Mass (kg)	Interval (days)	Distance (km)
M	15/04/89	L	12.5	0.046	17/03/97	R	80.0	11.0	2892	10.0
M	28/07/88	L	13.1	0.044	26/08/96	R	83.0	7.0	2948	18.0
M	22/11/87	L	17.2	0.087	02/09/96	R	90.5	17.0	2835	15.0
M	17/04/92	L	13.9	0.054	14/07/97	R	71.5	7.7	1915	8.5
M	06/12/89	L	36.1	1.10	19/11/99	L	80.0	12.0	3633	3.5
M	04/12/87	L	52.5	3.5	13/02/98	R	105.0	26.0	3719	5.0
M	03/12/87	L	61.9	5.0	02/11/97	R	104.0	32.0	3620	9.25
M	27/09/89	L	69.0	7.5	19/11/99	L	82.5	12.0	3701	0.0
M	20/10/94	R	81.0	11.5	29/09/99	R	107.0	38.0	1805	0.0
M	08/11/94	R	84.0	15.0	29/09/99	R	106.0	31.0	1825	1.3
M	08/11/94	R	98.5	25.0	07/10/99	R	101.0	22.0	1795	1.5
M	20/10/94	R	85.5	14.0	26/09/99	R	108.0	-	1801	0.0
M	05/10/89	L	104.1	27.0	10/09/99	R	110.0	28.5	3625	5.0
M	06/01/88	L	113.5	31.0	06/08/93	L	114.0	-	2005	1.5
F	10/12/89	L	19.4	0.114	19/11/99	L	72.0	9.0	3628	7.5
F	20/10/94	R	60.8	6.0	30/09/99	R	79.0	11.0	1805	0.0
F	02/12/87	L	66.9	6.0	05/08/93	L	79.0	9.0	2068	1.5
F	21/02/87	L	66.9	6.0	07/08/93	L	79.0	9.0	2356	2.15
F	21/11/87	L	40.2	1.4	27/01/01	L	85.0	-	5136	4.0
F	05/10/89	L	79.2	14.0	29/01/01	L	84.0	-	4129	0.0
F	19/09/89	L	78.7	11.0	07/11/94	R	79.0	13.0	1873	7.1

Effect of Movement on Vulnerability to Hunting

Local hunters informed us that carcasses were generally left near the site of capture, and hunting sites were identified from carcasses and skeletons left on the banks of rivers and lakes. Four hunting sites were in the lake area and 18 in the river area. Between 1989 and 1990, we found plastic numbered tags that we used to mark caimans at the hunting sites. The caimans had been marked in the lake area 515–1065 days (mean=794 days, SD=229) before being found at river hunting sites. All caimans killed were males and their sizes at original capture were greater than 60 cm SVL (Table 2). The distances moved by the caimans from their initial capture sites to where they were killed by hunters varied from five to 11 km (mean=8.0 km, SD=2.4).

Radio-telemetry

The pattern of water level fluctuations in the river channel was generally similar in 1992–2001, with low levels between August and December. The months between August and December were dry in all years, but in other months, water level varied between years. The monthly movement rate (MR) of the caimans with radio-transmitters was not related to water level (L) (MR=74.267–0.566×L, R^2=0.107, n=26, P=0.103). The mean movement rate of the caimans was only 46.7±66.9 m/day. There was no significant difference between movement rates of males and females (Kruskal Wallis test; KS=7.8, P=0.253, SD=6). The body condition of the caimans with radios was not related to monthly movement rate (r^2=0.008, P=0.686, n=22).

The area used by 47 caimans (Table 3) tracked for up to 436 days in the river area varied from 2 to 1650 ha (mean=153, SD=299). About 25% of the variance in the size of the area used (A – ha) was accounted for by the multiple regression, which included snout-vent length of the caiman (SVL – cm), log interval (logI – days), and sex of the caiman (S, dummy variable coded 0,1) (A=-582–2.299×SVL+198.6×logI+4.774×S,R^2=0.254;$F_{3,42}$=4.89, P=0.005). However, only time of tracking (P≤0.001) contributed significantly to the model. There was little evidence of an effect of SVL (P=0.549) or sex (P=0.965).

Although there is a large scatter of points, both LOWESS regression (Fig. 6 – broken line) and log-linear regression (Fig. 6 – solid line) indicate that estimates of area used increased slowly after about 225 days. One individual covered a large area (1649 ha) in 246 days, and was an outlier in the analyses. However, removal of this individual had little effect on the position of the lines, and did not affect the statistical conclusions. The mean of the maximum distances between records for caimans tracked for more than 225 days was 4.6 km (min.=1.0, max.=9.8).

During the period of experimental hunting, 10 males were monitored in the experimental blocks in the river area over periods of 249–436 days (Table 4). The area used by the 10 caimans varied from 50 to 777 ha (mean=348, SD=255). However, the mean area used by the five caimans in the hunted blocks not was significantly different from the mean area used by five caimans in the control blocks (ANOVA, $F_{2,7}$=2.32, P=0.171).

DISCUSSION

Little is known about dispersal in wild populations of vertebrates (Horn, 1984), and one reason is the difficulty of marking young individuals and recapturing them as adults. This difficulty is especially great for crocodilians, which have long life spans and can move great distances. The individual *Caiman crocodilus yacare* in this study moved not only inside the lake and river areas, but also between them. We were able to record movements of some individuals from soon after hatching to adulthood, but only by marking more than 5000 animals and maintaining capture effort over 15 years.

The distances moved by hatchling *C. c. yacare* varied little between the first and second years of life, when they tended to remain near nests. In the first year of life, some individuals moved distances of up to 6 km in the lake area and up to 1 km in the river area. However, we have no evidence of hatchlings moving from the lake to the river area, or vice versa, during the first two years of life.

Hatchlings in the lake area remained together with an adult for up to 11 months. In the northern Pantanal, the majority of hatchling *C. c. yacare* remained within 200 m of their nests over a period of six months, and were attended by females (Cintra, 1989). Da Silveira *et al.* (1997) reported that clutches of *C. c. crocodilus* are also relatively sedentary in the first months of life.

In this study, individual *C. c. yacare* moved extensively throughout the year. Although there was a significant relationship between water level and distance moved by animals recaptured over periods of up to one year, the effect of water level was not apparent in the data for animals monitored by radio-telemetry. In the dry period, caimans remained concentrated in the remaining pools. However, they regularly moved between pools (Campos *et al.*, 2003). Why caimans move between pools in groups is unknown, but may be related to

TABLE 2. Sizes of caimans marked in the lake area and found dead at hunting sites in the river area, and distance from where they were marked.

Date of capture	SVL (cm)	Mass (kg)	Date of death	Interval (days)	Distances (km)
24/03/87	60.0	-	24/02/90	1065	11.0
13/03/87	92.4	13.0	13/01/90	1035	6.1
24/04/87	80.0	-	23/12/89	970	9.5
05/12/87	101.0	27.0	08/12/89	733	9.25
31/07/87	104.3	24.0	31/01/89	545	5.5
20/08/87	59.0	5.0	20/01/89	515	5.0
02/01/88	64.2	5.4	08/12/89	700	9.5

TABLE 3. Sizes and distances moved by caimans tracked by radio-telemetry.

SVL (cm)	Mass (kg)	Body condition	Sex	Interval (days)	Area used (ha)	Maximum distance moved (km)
95.2	27.0	0.303	M	114	42.0	1.75
82.4	16.0	0.202	F	123	22.3	1.35
71.1	10.0	0.163	F	246	1649	9.80
83.4	16.0	0.167	F	239	32.2	1.00
80.0	12.5	0.042	F	144	7.0	0.30
96.0	20.0	-0.021	M	144	19.0	1.00
103.0	22.0	-0.132	M	216	728.2	7.50
99.0	22.0	-0.016	M	140	63.0	2.75
71.0	9.0	0.062	F	126	42.3	0.50
74.0	10.0	0.046	F	125	6.0	0.00
115.0	37.5	0.079	M	159	50.0	1.50
85.0	16.0	0.111	M	116	96.2	1.90
102.0	24.0	-0.016	M	60	8.120	2.40
108.0	32.0	0.104	M	75	48.4	1.75
76.5	10.0	-0.051	F	157	17.1	1.65
72.5	8.5	-0.056	F	47	14.0	2.25
80.5	12.0	-0.017	F	102	36.5	2.75
68.0	9.0	0.188	F	83	261.6	7.50
84.0	14.0	0.012	F	185	41.5	6.75
79.0	11.5	-0.005	F	169	168.4	5.00
87.5	18.0	0.144	F	82	172.5	2.50
74.0	9.0	-0.059	F	48	2.0	0.75
105.0	24.0	-0.101	M	96	15.4	6.75
99.0	21.0	-0.062	M	397	90.0	5.00
111.0	28.5	-0.091	M	399	86.2	3.70
99.5	25.0	0.097	M	398	50.0	2.50
117.0	36.0	-0.012	M	395	417.0	3.50
108.0	26.0	-0.103	M	249	426.0	2.70
97.0	20.0	-0.052	M	335	210.6	2.50
93.5	18.5	-0.022	M	249	378.4	5.00
114.0	35.0	0.036	M	436	727	7.50
102.0	22.5	-0.081	M	335	777	8.75
112.5	32.5	0.001	M	397	313	3.75
58.0	4.0	-0.158	M	37	11.5	0.00
111.0	28.0	-0.109	M	36	11.5	0.50
99.0	22.0	-0.016	M	62	11.5	0.00
74.0	8.0	-0.177	F	42	11.5	0.00
89.0	12.0	-0.311	M	39	11.5	0.00
60.0	5.0	-0.034	F	35	11.5	0.00
75.0	8.5	-0.155	F	44	3.0	0.00
87.5	14.0	-0.107	F	38	3.0	0.00
80.0	11.0	-0.086	F	38	3.0	0.00
82.5	12.0	-0.089	F	38	3.0	0.00
79.5	14.0	0.173	F	38	72.2	2.50
78.5	11.0	-0.031	F	42	3.0	0.00
85.0	14.0	-0.022	F	38	3.0	0.00
63.5	6.0	-0.017	M	57	11.5	0.00

reproductive behaviour or feeding habits (Coutinho *et al*., 2000; Campos, 2003).

There was large individual variation in movement patterns for both sexes in *C. c. yacare*. Some apparently moved short distances or returned to the same water body after many years, and others were recorded to move great distances in short periods. Caimans undertook extensive movements in both the lake and river areas. The distributions of the distances moved by males and females were similar in the short and long

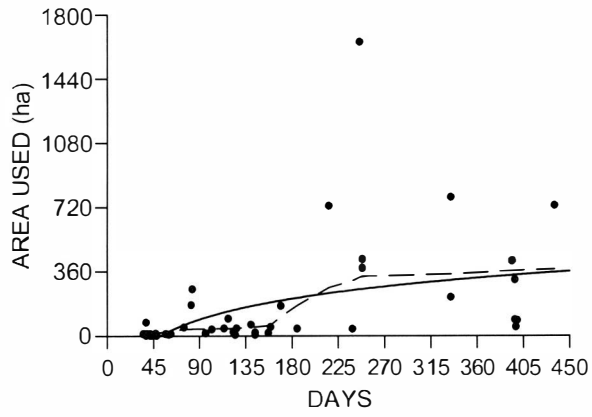

FIG. 6. Areas used by 46 caimans with radio transmitters in the river area in relation to the period the caimans were monitored. The line broken line is a LOWESS regression (tension = 0.5), and the solid line represents a least-squares linear-log regression.

terms. In periods of up to one year, caiman moved distances of up to 9.8 km. In the northern Pantanal, the maximum distance moved in the dry season by *C. c. yacare* in an interval of six months was 9.4 km (Schaller & Crawshaw, 1982). However, Ouboter & Nanhoe (1988) found that most individual *C. c. crocodilus* in their study area in Suriname were sedentary and the largest distance moved in one year was 3 km. In our study, male caimans were recorded moving distances of up to 18 km, and females up to 14 km, in the long term. Movements over distances greater than these would have taken caimans out of the study area, so our data probably underestimate the degree of movement.

Although hatchling caimans tended to remain together close to the nesting site during the first two years after hatching, individuals of SVL>40 cm moved extensively within and between habitats. For individuals with SVL>40 cm, smaller individuals were recorded moving larger distances than larger individuals, and small individuals may have moved greater distances than those recorded, as more extensive movements would have taken them out of the study area. Distances moved in the long term by caimans with SVL>52 cm were similar to distances moved by larger individuals in the short term, indicating that individuals may establish relatively fixed home ranges at about this size.

The pattern of movement of some species of vertebrates is related to density (Hansson, 1991), and increase in disturbance could cause increased movement. However, the reduction in population density and the disturbance caused by hunting in the experimental blocks did not result in differences in the area used by the five males monitored by radio telemetry within hunted blocks in relation to the five males monitored in areas without hunting.

Our study area was around 50,000 ha, involved two ranches, and apparently was too small to study dispersal of caimans. The caimans moved from the lake area to the river area and, within the river area, moved from one river to another. This probably contributes to the low recapture rate of the caimans in the study area. However,

TABLE 4. Sizes of male caimans used to investigate movement in hunted and unhunted areas.

SVL (cm)	Mass (kg)	Interval (days)	Distance (km)	Area used (ha)	Hunted area
99.0	21.0	397	5.0	90.0	No
111.0	28.5	399	3.7	86.2	No
99.5	25.0	398	2.5	50.0	No
97.0	20.0	335	2.5	210.6	No
114.0	35.0	436	7.5	727.0	No
93.5	18.5	249	5.0	378.4	Yes
117.0	36.0	395	3.5	417.0	Yes
108.0	26.0	249	2.5	210.6	Yes
102.0	22.5	335	8.75	777.0	Yes
112.5	32.5	397	3.75	313.0	Yes

movement over large distances between habitats can be an advantage in sustained-use programmes, if populations of caimans in areas that are not hunted can sustain recruitment to populations in hunted areas. In the controlled harvest programme in Venezuela, monitoring is done over units much larger than individual ranches and quotas are determined on a regional basis (Velasco *et al.*, 2003). In contrast, individual ranches are monitoring units in the egg-collection program in the Pantanal (Coutinho *et al.*, 1998). The results of this study indicate that ranches are not large enough to be considered independent management units in the Pantanal, and that movement between ranches should be considered when allocating quotas to individual ranches.

It has been suggested that hunted populations of South American vertebrates operate as source–sink systems, with immigration from lightly hunted areas maintaining stocks in more heavily hunted regions (Bodmer, 1999). Information from hunters, and the distribution of hunting sites, indicates that hunting was more intensive in the river area than the lake area. This could be because the caimans tend to be larger in the river area, because there is less available water in the dry season and the caimans become more concentrated in the river area, because of the difficulty of hunting in the heavily vegetated lakes, or a combination of these factors.

Based on the size distribution of the populations, the number of hatchlings produced is much greater in the lake area than in the river area, despite the smaller average size of the females (Campos & Magnusson, 1995). However, movement of caimans from the lake area to the river area is frequent; most of the caimans marked in the lake area that were recaptured over periods of ≥5 yrs moved to the river area. Four of the five males that were marked as hatchlings in the lake area were recaptured as adults in the river area 6–9 years later, 8–18 km from where they were marked. Other individuals marked as hatchlings may have dispersed larger distances, but these would have moved out of the study area.

The combination of light hunting, extensive reproduction and frequent dispersal probably result in the lake area being a source for the hunted population. In con-

trast, intensive hunting and low reproductive rate probably mean that the river population is a sink, and would not have been able to sustain the intensive hunting of the 1980s (Mourão *et al.*, 1996), if it had been isolated from the lake area. Some other hunted populations of crocodilians also appear to represent source-sink systems, with most reproductive individuals in areas that are not readily accessible to hunters (e.g. *Alligator mississippiensis* – Joanen & Mc'Nease, 1987; Elsey & Kinler, 2004; *Melanosuchus niger* – Da Silveira, 2001).

Whatever the specific roles of Nhumirim and Campo Dora Ranches in the dynamics of local populations of caimans, this study has shown that individual *Caiman crocodilus yacare* undertake extensive movements seasonally, annually and over the life span of individuals. These movements cover areas much larger than the average size of ranches in the Pantanal, so individual ranches should not be considered autonomous units in management plans for the Pantanal caimans.

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REFERENCES

Bodmer, R. E. (1999). Uso sustentable de los ungulados amazônicos: implications par las areas protegidas comunales. In *Manejo y Conservation de Fauna Silvestre en América Latina*, 51-58. Fang, T. G., Montenegro, O. L. & Bodmer, R. E. (Eds). New York: Columbia University Press.

Bustard, H. R. & Choudhury, B. C. (1979). Long distance movement by a saltwater crocodile (*Crocodylus porosus*). *British Journal of Herpetology* **6**, 87-92.

Bustard, H. R. & Singh, L. A. K. (1983). Movement of wild gharial, *Gavialis gangeticus* (Gemini) in the River Mahanadi, Orissa, (India). *British Journal of Herpetology* **6**, 287-291.

Campos, Z. (2003). *Caiman crocodilus yacare*. Food-related movement. *Herpetological Review* **34**, 141-141.

Campos, Z., Coutinho, M. & Magnusson, M. (2003). Terrestrial activity of caiman in the Pantanal, Brazil. *Copeia* **2003**, 628-634.

Campos, Z. & Magnusson, W. (1995). Relationship between rainfall, nesting habitat and fecundity of *Caiman crocodilus yacare* in the Pantanal, Brazil. *Journal of Tropical Ecology* **11**, 351-358.

Campos, Z., Mourão, G., Coutinho, M. & Abercrombie, C. (1995). Night-light counts, size structure, and sex ratio in wild populations of yacare caiman (*Caiman crocodilus yacare*) in the Brazilian Pantanal. *Vida Silvestre Neotropical* **4**, 46-50.

Caughley, G. (1977). *Analysis of Vertebrate Populations*. London: John Wiley & Sons.

Cintra, R. (1989). Maternal care and daily pattern of behavior in a family of caimans, *Caiman yacare* in the Brazilian Pantanal. *Journal of Herpetology* **23**, 320-322.

Cooper-Preston, H. (1992). *Geographic Variation in the Population Dynamics of* Crocodylus johnstoni *(Krefft) in Three Rivers in the Northern Territory, Australia*. Ph.D. dissertation, University of New England, Armidale, Australia.

Coutinho, M. (2000). *Population Ecology and the Conservation and Management of* Caiman yacare *in the Pantanal, Brazil*. Ph.D. dissertation, University of Queensland, Brisbane, Australia.

Coutinho, M. & Campos, Z. (1996). Effect of habitat and seasonality on the densities of caiman in Southern Pantanal – Brazil. *Journal Tropical Ecology* **12**, 741-747.

Coutinho, M., Campos, Z., Bampi, I. & Dal'Ava, F. (1998). Preliminary report for the management system of yacare caiman in the Pantanal: a proposal for future research and the development of a monitoring system for wild population subjected to nest harvests. *Ciência e Cultura* **50**, 60-64.

Coutinho, M., Campos, Z., Cardoso, F., Martinelli, P. & Castro, A. (2000). Reproductive biology and its implication for management of caiman *Caiman yacare* in the Pantanal wetland, Brazil. In *Crocodilian Biology and Evolution*, 229-243. Gordon, G., Seebacher, F. & Franklin, C. E. (Eds). Chipping Norton, Australia: Surrey Beatty & Sons.

Da Silveira, R. (2001). Monitoramento, crescimento e caça de jacaré-açu (*Melanosuchus niger*) e de jacaré-tinga (*Caiman crocodilus crocodilus*). Ph.D. dissertation INPA/UA, Manaus, Brazil.

Da Silveira, R., Magnusson, W. E. & Campos, Z. (1997). Monitoring of distribution, abundance and breeding areas of *Caiman crocodilus crocodilus* and *Melanosuchus niger* in the Anavilhanas archipelago, Central Amazonia, Brazil. *Journal of Herpetology* **31**, 514-520.

Elsey, R. & Kinler, N. (2004). Louisiana's alligator program: adapting management as populations recover and risk of unsustainable use decreases. In *Crocodiles*, 92-101. Proceedings of the 17th Working Meeting of the Crocodiles Specialist Group of the Species Survival Commission of IUCN. Darwin: IUCN.

Hansson, L. (1991). Dispersal and connectivity in metapopulations. In *Metapopulation Dynamics: Empirical and Theoretical Investigations*, 89-103. Gilpin, M. E. & Hanski, I. (Eds). London: Academic Press.

Hayne, D. W. (1949). Calculation of size of home range. *Journal of Mammalogy* **30**, 1-18.

Horn, H. S. (1984). Some theories about dispersal. In *Dispersal and Migration*, 55-62. Lidicker, Jr, W. Z. & Caldewell, R. C. (Eds). USA: Hutchinson Ross Publishing Company.

Hutton, J. (1989). Movements, home range, dispersal and separation of size classes in Nile crocodiles. *Animal Zoology* **29**, 1033-1049.

Joanen, T. & Mc'Nease, L. (1987). The management of alligators in Louisiana, USA. In *Wildlife Management: Crocodiles and Alligators*, 33-42. Webb, G. W., Manolis, S. C. & Whitehead, P. J. (Eds). Chipping Norton, Australia: Surrey Beatty & Sons.

Lang, J. (1987). Crocodilian behaviour: implications for management. In *Wildlife Management: Crocodiles and Alligators*, 273-294. Webb, G. W., Manolis, S. C. & Whitehead, P. J. (Eds). Chipping Norton, Australia: Surrey Beatty & Sons.

Montague, J. J. (1983). Influence of water level, hunting pressure, and habitat type on crocodile abundance in the Fly River drainage, Papua New Guinea. *Biological Conservation* **26**, 309-339.

Mourão, G. M., Campos, Z., Coutinho, M. & Abercrombie, C. (1996). Size structure of illegal harvested and surviving caiman (*Caiman crocodilus yacare*) in the Pantanal, Brazil. *Biological Conservation* **75**, 261-265.

Mourão, G., Coutinho, M., Mauro, R., Campos, Z.,Tomás, W. & Magnusson, W. (2000). Aerial surveys of caiman, marsh deer and pampas deer in the Pantanal Wetland of Brazil. *Biological Conservation* **92**, 175-183.

Munõz, M. C.& Thorbjarnason, J. (2000). Movement of captive-released Orinoco crocodiles (*Crocodylus intermedius*) in the Capanaparo river, Venezuela. *Journal of Herpetology* **34**, 397-403.

Ouboter, P. E. & Nanhoe, L. M. R. (1988). Habitat selection and migration of *Caiman crocodilus crocodilus* in a swamp and swamp-forest habitat in Northern Suriname. *Journal of Herpetology* **2**, 283-294.

Pooley, A. C. & Gans, C. (1976). The Nile crocodile. *Scientific American* **234**, 114-124.

Schaller, G. B & Crawshaw, P. G. (1982). Fishing behavior of Paraguayan Caiman (*Caiman crocodilus*). *Copeia* **1982**, 66-72.

Soriano, B. A. (1997). *Boletim Agrometeorológico: 1989–1996*. Corumbá, MS, Brazil: Empresa Brasileira de Pesquisa Agropecuária – CPA Pantanal.

Tucker, A. D., Limpus, C. J., McCallum, H. I. & McDonald, K. R. (1997). Movement and dispersal of the Australian freshwater crocodile (*Crocodylus johnstoni*) in the Lynd River, Queensland. *Wildlife Research* **24**, 379-396.

Velasco, A., Colomina, G., De Sola, R. & Villarroel, G. (2003). Effects of harvests on wild populations of *Caiman crocodilus crocodilus* in Venezuela. *Interciencia* **28**, 544-548.

Webb, G. J. W. & Messel, H. (1978). Movement and dispersal patterns of *Crocodylus porosus* in some rivers of Arnhem Land, Northern Australia. *Australian Wildlife Research* **5**, 263-283.

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AGE, BODY SIZE AND GROWTH OF *LACERTA AGILIS BOEMICA* AND *L. STRIGATA*: A COMPARATIVE STUDY OF TWO CLOSELY RELATED LIZARD SPECIES BASED ON SKELETOCHRONOLOGY

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Age and growth in *Lacerta agilis* (ssp. *L. a. boemica*) and a closely related sympatric species *L. strigata* from the eastern North Caucasus, Russia were assessed by skeletochronology and back-calculation methods. We examined 320 specimens from one lowland, two submontane (both species), and two mountain (*L. agilis*) localities. Age structure, back-calculated snout-vent length (SVL) at hatching and subsequent hibernations, and asymptotic SVL were studied for sexual dimorphism, altitudinal variation and interspecific differences. Pattern of resorption of growth layers in bone and its possible effects on growth inferences from skeletochronological data were also considered. The back-calculated SVLs showed a good conformity to comparable field data. Mean and maximum SVL at the first hibernation clearly decreased with altitude. Within the same localities, these parameters were consistently higher in *L. agilis* than in *L. strigata*. Between the 1st and 2nd hibernations (the period of the highest increment in SVL in all study populations), *L. strigata* grew faster than the syntopic *L. agilis*. In the lowland locality, females of both species tended to grow slower than males between the 1st and 2nd hibernations, while at higher elevations they exhibited lower SVL increments than the males between the 2nd and 3d hibernations. This pattern, along with occurrence of gravid yearlings in the lowland locality (but not in the other sites), suggests an earlier onset of reproduction in the lowland populations compared to those from higher elevations. Asymptotic SVLs in the study populations tended to be larger in males than in females. In *L. agilis* these sexual size differences (SSD) varied among populations, being quite strong in the lowland site and negligible at the highest locality. The mountain populations (960 and 1900 m a.s.l.) of *L. a. boemica* exhibited higher mean age and longevity than the lowland and submontane populations (20-600 m a.s.l.) of both species; however, no clear altitudinal trend was found for adult SVL. Much of the variation revealed in this study, including the interlocality differences in SSD, can be related to the length of activity season, in line with recently published theoretical models and experimental studies stressing the role of proximate factors.

Key words: altitudinal variation, asymptotic size, life-history, lizards, sexual size dimorphism

INTRODUCTION

In recent decades lizards have become model organisms for studying factors determining variation of life-history and demography traits within and among species (Pianka & Vitt, 2003). An advanced theoretical and methodological framework has been developed in this field providing estimates of the relative importance of evolutionary constraints (Dunham *et al.*, 1988; Bauwens & Diaz-Uriarte, 1997), genetic adaptation and phenotypic plasticity (e.g. Grant & Dunham, 1990; Adolph & Porter, 1993, 1996; Niewiearowski & Roosenburg, 1993; Qualls & Shine, 2000; Lorenzon *et al.*, 2001). These studies have mostly been confined to a small set of populations and taxa (North American iguanids, a few species of Australian skinks and West European lacertids). The problem is that both mark-recapture and experimental studies – the main tools used to obtain data on growth and longevity – are very time-consuming.

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A reasonable alternative tool to obtain such data arise from life-history investigations based on counting and measuring growth layers in the bone or other hard tissues (Mina & Klevezal, 1970). The formation of these layers reflects the seasonal changes of the growth rate of an animal. This method, known among herpetologists as skeletochronology (Castanet *et al.*, 1977), provides not only accurate age determination in reptiles and amphibians (Smirina, 1972; Castanet *et al.*, 1977; Castanet & Smirina, 1990) but also a quantitative estimation of the pattern of bone growth (Castanet & Baez, 1991; Bruce *et al.*, 2002; Sinsch *et al.*, 2002; etc.). Due to a generally high correlation between the bone thickness and body size, body size at the time of formation of a corresponding growth mark can be back-calculated from the size of the growth mark, current body size and current bone thickness. Such retrospective estimation of body size at specific ages is a common practice in fish studies (Francis, 1990). This procedure was also used on amphibians (e.g. Smirina, 1983; Marunouchi *et al.*, 2000), but,

with the exception of one study on turtles (Sergeev, 1937), it has not yet been applied to reptiles.

The objective of the present study was to comparatively examine altitudinal variation and sex differences for age structure, asymptotic body size and growth pattern in two related syntopic lizard species from the Caucasus. The data were obtained from preserved specimens using skeletochronology and back-calculation methods. As the back-calculation technique had never before been applied to squamate reptiles, particular attention was given to the methodological problems that can arise through inferences about body growth from skeletochronological data.

MATERALS AND METHODS

STUDY SPECIES

L. agilis and *L. strigata* are medium-sized, diurnal, insectivorous lizards of the family Lacertidae. They are closely related (although non-sister) species belonging to the subgenus *Lacerta s. str.* (= *Lacerta I* group *sensu* Arnold, 1989). *L. agilis* occupies a larger part of the temperate zone of the Palaearctic from southern England and the Pyrenees in the west to the Baikal Lake in the east (Bischoff, 1988), and *L. strigata* inhabits the eastern Caucasus and adjacent parts of Turkey and Iran (Darevskij, 1984). In the eastern Caucasus, *L. agilis* and *L. strigata* are broadly sympatric and often coexist in the same habitats (Roitberg, 1982), providing an opportunity for a comparative study of two species in a common range of environmental conditions. Daghestan (the south-eastern North Caucasus) seems particularly promising for such studies due to considerable landscape/climatic heterogeneity within a small area. Moreover, *L. agilis* is represented here by a very peculiar subspecies, *L. a. boemica* whose coloration, scalation and body proportion features make it the most similar to the other related species (*L. strigata*, *L. viridis*, etc.) and the most distinct phenetically, compared to the other subspecies of *L. agilis* (Roitberg, 1987). According to a recent molecular-genetic study (Kalyabina *et al.*, 2001) *L. a. boemica* also exhibits a strong separation from the rest of the species occupying a basal position in the species phylogeny.

STUDY SAMPLES AND COLLECTION SITES

Our study material comprised 540 specimens from five *L. a. boemica* populations and three *L. strigata* populations; 320 of them (all adults and the largest subadults) were used for skeletochronological analysis (Table 1). No animals were sacrificed for this study. We used only preserved specimens that had already been collected for other purposes (Roitberg, 1982, 1987, 1989, etc.). The specimens originate from five localities (sites) in the lowlands (Kostek), submontane (Sergokala, Khuchni) and montane (Termenlik, Kuli) regions of Daghestan (Appendix 1). In Kostek, Sergokala and Khuchni the two species live syntopically and were sampled from the same collection sites. In Termenlik and Kuli *L. strigata* did not occur.

AGEING AND MEASURING GROWTH MARKS

The annual pattern of growth layer deposition in bone was validated experimentally for various reptilian and amphibian species living in temperate climates (Smirina, 1972; Castanet *et al.*, 1993), including the lizard *Lacerta bilineata* (=*L. viridis*) (Castanet *et al.*, 1993). Since *L. bilineata* is closely related to our study species (Arnold, 1989) and the winters in the North Caucasus are not milder than in West Europe, we are greatly confident that the number of resting lines (=lines of arrested growth, or LAGs - Castanet *et al.*, 1977) corresponds to the number of hibernations experienced by the individual. The LAGs were counted on transverse sections of the middle part of femur diaphysis, the growth pattern was assessed by measuring bone diameters limited by consecutive LAGs (Fig. 1). Each femur bone was decalcified in 5% nitric acid solution, cross sectioned (20-25 μm thick) with a freezing microtome and stained with Ehrlich haematoxylin. Diameters limited by consecutive LAGs and by the outer bone margin ($D_1, D_2 \ldots D_n$, D) were measured with ocular-micrometer under a light microscope. As the contours of the bone sections deviate from a true circle, means of the minimal and maximal diameter (Fig. 1), measured in three sections from every specimen, were used to estimate the bone width at the time of a LAG formation and at capture. The word "diameter" is used here as a workable quantification of bone thickness and not as a strict geometric term.

For nearly all the femurs examined, LAGs were well defined providing precise age estimation. Additional and double lines (Castanet & Smirina, 1990) did occur but were easily recognisable. Only two femurs gave some difficulties in age estimation but the possible error margin did not exceed 1 year.

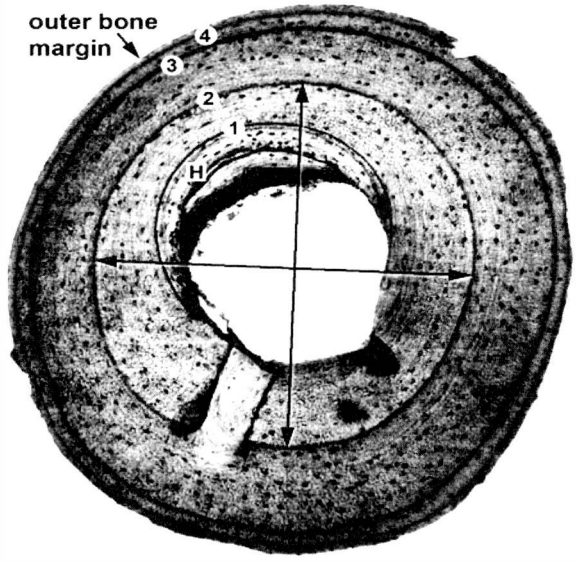

FIG. 1. Cross section of the femur diaphysis of *Lacerta agilis boemica* (adult male from Kuli). Ehrlich hematoxylin; magnification: ob. 6.3, oc. 3.2. H - hatching line; 1-4 - LAGs (= resting lines) of consecutive hibernations; arrows inside the section show the minimal and the maximal diameters of the bone limited by LAG2.

BACK-CALCULATION PROCEDURE

A high correlation between SVL and femur diameter (0.84-0.98 in our study samples – Roitberg & Smirina, 1995, 2005; see also Castanet & Baez, 1991; Arakelyan, 2002) made it reasonable to perform back-calculation of individual body length at the time of each LAG formation (hatching and subsequent hibernations). Different back-calculation formulae (BCFs) have been proposed and used for back-calculating body size from the size of a growth mark (Francis, 1990; see also Marunouchi *et al.*, 2000). The simplest BCF, the Dahl-Lea formula, is merely a proportion: $L_i = L_c(D_i/D_c)$, where L_i and L_c are snout-vent lengths at the time of formation of the i^{th} LAG and at capture; D_i and D_c are the bone diameters limited by LAG_i and by the outer bone margin, respectively. The other BCFs also incorporate, along with L_c, D_i and D_c, parameter(s) of a regression of L on D (or D on L) in the corresponding population (Francis, 1990). The only but quite powerful validation study made on an ectotherm tetrapod (the frog *Rana japonica*) showed that all eight BCFs tested tended to overestimate the actual SVL at the previous capture (Marunouchi *et al.*, 2000). Surprisingly, the simple Dahl-Lea BCF provided the best estimation (mean deviation +0.6, SD 2.8 mm) (Marunouchi *et al.*, 2000). This BCF was used in the present study.

Another advantage of the Dahl-Lea BCF is that it does not require calculation of the regression parameters. A reliable estimation of these parameters can encounter substantial problems as the corresponding sample should not only be large enough, but should also cover the entire population size range (if the latter is not the case, the estimates might become biased: Ricker, 1973; Francis, 1990). These requirements could be fulfilled by pooling conspecific samples from different localities. However, in the study species the relationship between the body length and bone thickness did differ among localities (Roitberg & Smirina, 2005) making locality pooling inappropriate.

ESTIMATING ASYMPTOTIC LENGTH

Using nonlinear regression techniques, two asymptotic growth models were fitted to our age-size data for each combination of population and sex: the von Bertalanffy model $L_t=A-(A-L_0)e^{-k(t-to)}$ and the logistic-by-length model $L_t=A/[1+(A/L_0-1)e^{-k(t-to)}]$, where e is the base of the natural logarithm, t is age (number of growing seasons experienced), t_0 and L_0 are age and length at the start of the growth interval under study, A is the (average) asymptotic SVL (in mm), k is the characteristic growth rate. These two models for linear growth are the ones most commonly encountered in studies on reptiles (Andrews, 1982; James, 1991; Kratochvil & Frynta, 2002). Taking into account the scarcity of our data on the size at hatching (SVL_0) which is usually taken as L_0 (Andrews 1982; Kratochvil & Frynta, 2002), we chose mean SVL_1 and $t_0 = 1$ (length and age at the first hibernation) as initial values of the above growth models. Doing so might have an additional advantage since the time of entering the first hibernation is probably less variable than the hatching time, at least within populations.

In most of the samples, the von Bertalanffy model explained a slightly higher percentage of variance than the logistic model, and so was used to estimate the asymptotic SVL for all sites.

As sample estimates of the growth curve parameters can be sensitive to the structure of original data (see Discussion), we also used another estimate of the asymptotic body length, namely the 80 percentiles of the SVL distributions of adult animals (Brown *et al.*, 1999). In a mark-recapture study on the agamid lizard, *Agama impalearis*, this simple statistic exhibited a good conformity with growth-based estimations (Brown *et al.*, 1999).

STATISTICAL ANALYSIS

Operational units of statistical comparisons in this study were samples of specimens of the same species, the same sex and collected from the same locality. We designated these as study samples. Subsamples of individuals collected in different years were pooled for all analyses.

All data were tested for normality (Shapiro-Wilk *W*-test) and for homogeneity of variances (Levene test, Liliefors). Both parametric and non-parametric statistics were used for the analysis depending on the distribution type of the variable (Sokal and Rohlf, 1995). For multiple comparisons, we considered only those individual differences, which remained significant ($P<0.05$) after the sequential Bonferroni adjustment (Rice, 1989). We used SPSS 11.0 for all the analyses.

TABLE 1. Study sites and sample sizes. Values shown are the number of specimens used for skeletochronology, with overall sample size in parentheses.

Locality	Capture dates	*L. a. boemica*		*L. strigata*	
		males	females	males	females
1. Kostek	April-September 1984-85	24 (41)	30 (45)	26 (37)	21 (28)
2. Sergokala	11-14 June 1982	19 (35)	18 (34)	14 (31)	13 (37)
3. Khuchni	9-14 July 1985, 5-8 June 1986	28 (35)	25 (36)	26 (35)	24 (43)
4. Termenlik	April-September 1981-85	22 (23)	29 (32)		
5. Kuli	July 1992-93	10 (13)	13 (15)		

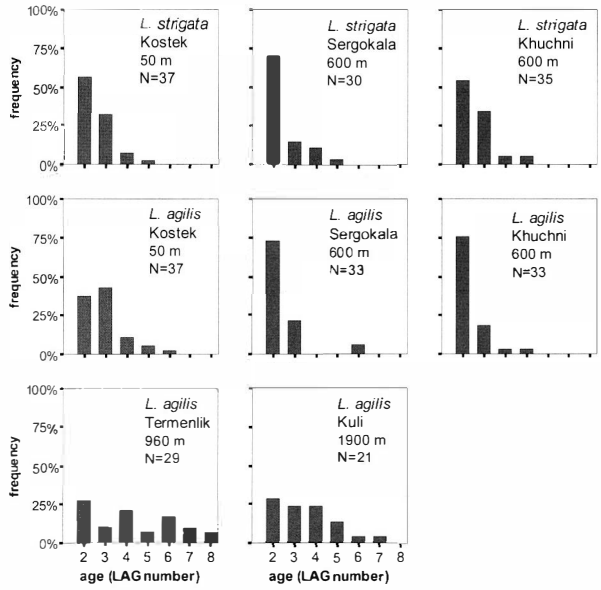

FIG. 2. Age (yr) distribution in adult samples of *L. a. boemica* and *L. strigata* from five different localities of the eastern North Caucasus. Locality elevations are given within the plots.

RESULTS

AGE STRUCTURE

In seven out of eight study populations, the proportion of yearlings to the total sample (yearlings + adults) was 43-56%. In the Kuli sample (the highest elevation) yearlings constituted only 22%. This proportion did not differ significantly between sexes. Age distributions in the samples of adults are presented in Fig. 2. Preliminary examination for sex differences had revealed only one significant value (*L. strigata*, Khuchni: P=0.012, Mann-Whitney test) in eight comparisons which became non-significant after Bonferroni correction. Therefore, sexes were pooled for further analysis. No significant differences were detected between syntopic populations of *L. a. boemica* and *L. strigata* within localities (P>0.05 in all three sites).

Two mountain *L. agilis* populations differed from the foothill and lowland populations of both species in having a large proportion of older animals and higher maximum age (Fig. 2). The latter were 7-8 years in the mountain localities and 5-6 years at the other study sites. Kruskal-Wallis tests revealed significant interpopulational differences for age structure in *L. agilis* (for all localities: χ^2=35.8, df=4, P<0.001; for the non-mountain localities: χ^2=9.9, df=2, P=0.007), but not in *L. strigata* (χ^2=2.0, df=2, P=0.38). Pairwise comparisons detected that the two mountain *L. agilis* populations did not exhibit significant difference from one another (Mann-Whitney test, P>0.2), but did differ from nearly all the other study populations. The lowland *L. agilis* population differed from both submontane *L. agilis* populations (P<0.02, Mann-Whitney test). To summarise, the age structure of the mountain *L. agilis* populations showed a clear shift to older ages as compared to the submontane populations, with the lowland *L. agilis* exhibiting an intermediate state.

PATTERN OF GROWTH MARKS RESORPTION

Resorption rates of the first two LAGs in the eight study populations, estimated as proportions of individuals in which the corresponding LAG diameters could not be measured, are given in Table 2 (as no consistent sex differences within populations were detected, males and females were combined for further comparisons). For LAG1, the resorption frequency was negligible for yearlings (0-10%) and increased dramatically for 2-yr-old lizards (up to 80-90% in some populations). However, animals that were at least 3-years old generally exhibited only a slight (if any) increase in the resorption frequency as compared to the 2-yr-olds from the same population (Table 2).

The resorption frequency for LAG1 in adults varied substantially between populations (Table 2). This variation was strongly associated with the interpopulational variation in mean LAG1 diameter (Spearman rank correlation coefficient between the two parameters, r_s=-0.857, P=0.007). As the mean LAG1 diameter tended to decrease with altitude in both species (Table 2), the LAG1 resorption frequency was lowest at the lowland locality and highest at moun-

TABLE 2. Percent of individuals with partial or complete resorption of the first two LAGs. Populations: *L. agilis* (1- Kostek, 2- Sergokala, 3- Khuchni, 4- Termenlik, 5- Kuli); *L. strigata* (11- Kostek, 12- Sergokala, 13- Khuchni).

Population	Mean D_1(mkm)	LAG 1								LAG 2	
		Yearlings		Age 2+		Age >2+		All adults		All adults	
		%	*n*	%	*n*	%	*n*	%	*n*	%	*n*
1	600	0.00	17	21.43	14	26.09	23	24.32	37	2.70	37
2	461	0.00	4	75.00	24	88.89	9	78.79	33	6.45	31
3	530	4.76	21	62.50	24	100.00	8	71.88	32	0.00	32
4	422	4.55	22	87.50	8	100.00	21	96.55	29	0.00	29
5	432	0.00	2	83.33	6	100.00	15	95.24	21	0.00	21
11	541	0.00	10	33.33	21	52.38	21	42.86	42	0.00	37
12	445	-	0	63.16	19	50.00	8	59.26	27	0.00	27
13	458	12.50	16	77.78	18	100.00	16	88.24	34	0.00	34

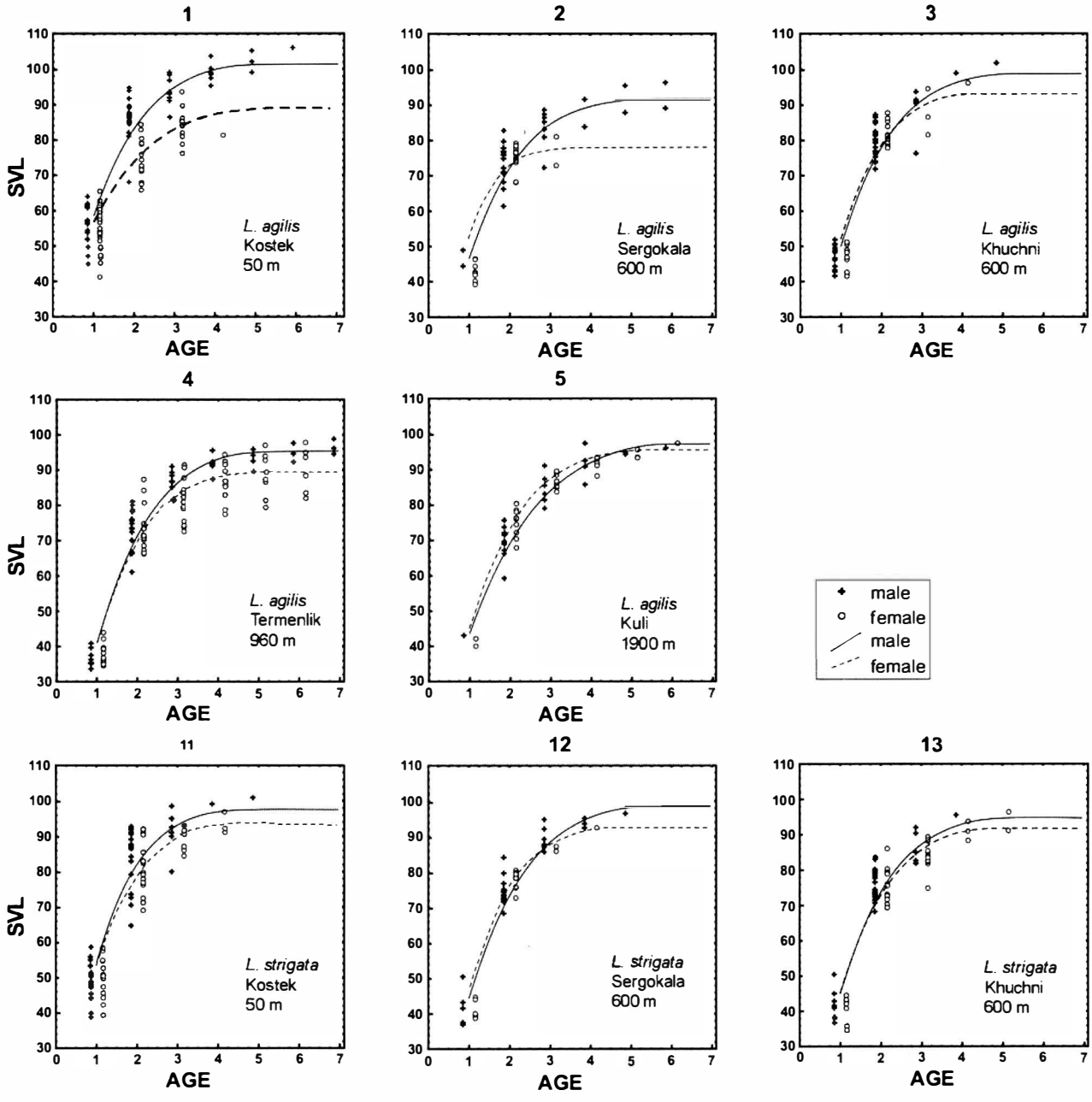

FIG. 3. Overall pattern of body growth in eight populations of *Lacerta agilis boemica* and *L. strigata*. Back-calculated SVLs (crosses, males; open circles, females) are plotted against age. Individual lizards occur more than once in this figure. Symbols for males and females are offset for clarity. Curves are best fit using the von Bertalanffy growth model. Locality elevations are given within the plots.

tain sites (Table 2). The resorption of LAG2 was extremely rare (Table 2) and no cases of resorption were revealed for the higher LAGs.

BACK-CALCULATED SVL

SVL at specific ages. Below we consider back-calculated snout-vent length (bc-SVL) at specific ages (the time of LAG formation in the femur) for lizards from the eight study populations. We assumed these SVLs to approximate the SVLs reached at the end of the corresponding activity seasons.

Back-calculated SVL at hatching (bc-SVL_0) was available for only a few individuals because the vast majority of the study specimens retained only a small (if any) portion of the neonate line in their femur bone. No sex and interlocality differences were found. Bc-SVL_0 was 30.4-37.2 (mean±SE: 34.5±0.39, n=20) for *L. a. boemica* and 32.6-35.9 (34.5±0.80, n=4) for *L. strigata*. These values are comparable with the data on hatchling SVL of the two taxa obtained in captivity by previous studies: *L. a. boemica*, Daghestan, 30-33 mm, n=23; *L. strigata*, Daghestan, 31-36 mm, n=29 (Roitberg, 1989); *L. a. boemica*, cf. Kabardino-Balkaria, 30-37 mm, n=37 (Warnecke, 2000); *L. strigata*, cf. Transcaucasia, 30-32 mm (Langerwerf, 1980).

Data on back-calculated SVLs at consecutive hibernations for the eight study populations (males and females separately) are compiled in Fig. 3. Following

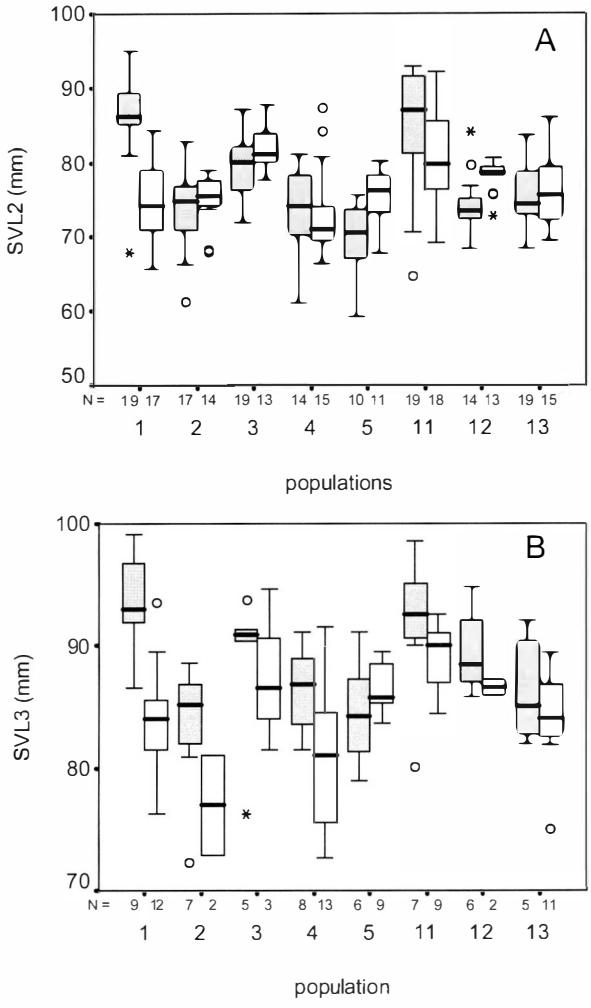

FIG. 4. Back-calculated SVL at the time of the second (A) and third (B) hibernation in *Lacerta agilis boemica* and *L. strigata* in the eastern North Caucasus. Populations: *L. agilis* (1- Kostek, 2- Sergokala, 3- Khuchni, 4- Termenlik, 5- Kuli); *L. strigata* (11- Kostek, 12- Sergokala, 13- Khuchni). The individual data are summarised as boxplots including the median (bold horizontal line), the 25th and 75th percentiles (box), range without outliers (vertical line), and outliers (observations located more than two interquartile ranges above or below the median) indicated as open circles or asterisks. Shaded figures are males and unshaded figures are females.

these data, the highest annual increment in SVL for all populations occurred between the 1st and 2nd hibernations (in yearlings) with progressively lower increments in subsequent activity seasons. Growth is, therefore, evidently asymptotic. Another related pattern is a clear separation between the SVL ranges of yearlings and adults (with the exception of the lowland population of *L. agilis* in which they nearly overlap) just at the beginning of the activity season (Fig. 3). Two samples (*L. strigata*, Sergokala and *L. agilis*, Kuli) showed only a small overlap between the 2-yr-olds and older animals, but in most populations these two age groups could not be identified by SVL.

Sex differences in SVL (sexual size dimorphism, SSD) showed the following patterns. No SSD was found for SVL at the first hibernation (bc-SVL_1). At the age of the second hibernation, SSD differed between the populations: it was male-biased (males are the larger sex) for *L. agilis* from Kostek and female-biased for *L. agilis* from Kuli and *L. strigata* from Sergokala (Mann-Whitney test, P=0.001, P=0.007, P=0.005 for the respective single tests; P<0.05 after the Bonferroni adjustment for eight simultaneous tests; Fig. 4A). No female-biased SSD were found for older ages (Fig. 4B). As the age-size relationships in the study samples (Fig. 3) became effectively linear when Age was log-transformed, an ANCOVA with SVL as the dependent variable, ln(Age) as the covariate and Sex as the factor was performed for each population to check for SSD consistency. In two *L. agilis* populations the effect of Sex was significant (after Bonferroni adjustment, P<0.001 for Kostek and P<0.05 for Termenlik) indicating a consistent male-biased SSD in adult animals.

Bc-SVL_1 exhibited clear differences between the species; size distributions of *L. a. boemica* are shifted towards higher values from those of sympatric *L. strigata* (Fig. 5; Mann-Whitney test, P<0.001 for Kostek and Khuchni, P=0.1 for Sergokala). Within each species, mean and maximum SVL_1 in the lowland locality (Kostek) were higher than in the submontane localities (Sergokala and Khuchni); for both species the Mann-Whitney test provided P<0.001 for the Kostek/Sergokala and Kostek/Khuchni comparisons. In *L. a. boemica*, there were differences between the mountain population of Termenlik on one hand and all the lowland and submontane populations on the other (Mann-Whitney test, P<0.001 for all three comparisons), with no significant differences being detected between the two mountain populations, Termenlik and Kuli (Mann-Whitney test, P=0.019, α= 0.05/10 = 0.005 after the Bonferroni correction for multiple comparisons among 5 samples). Thus, SVL_1 decreased with elevation in both study species.

Unlike for bc-SVL_1, the between-species and among-population comparisons for bc-SVL_2 showed no clear trend with altitude. In *L. agilis*, the males and females clearly exhibited different patterns of interlocality variation (Fig. 4A), that reflected a pronounced interlocality variation in the pattern of SSD (see above). No sex differences in the pattern of interlocality variation were found for *L. strigata*.

For bc-SVL_3, the pattern of interlocality variation was similar to that of bc-SVL_2, and in both species males from Kostek tended to be larger than those from other localities (Fig. 4B).

Asymptotic size. Asymptotic SVL and growth constant of the von Bertalanffy growth model were calculated for male and female samples of the eight study populations (Appendix 2; see also Fig. 3 for corresponding growth curves). Following the conservative approach of Schoener & Schoener (1978) we considered the observed differences between sample estimates of growth curve parameters as significant only if their 95% confidence intervals did not overlap. Sample estimates of asymptotic SVL in males were higher than

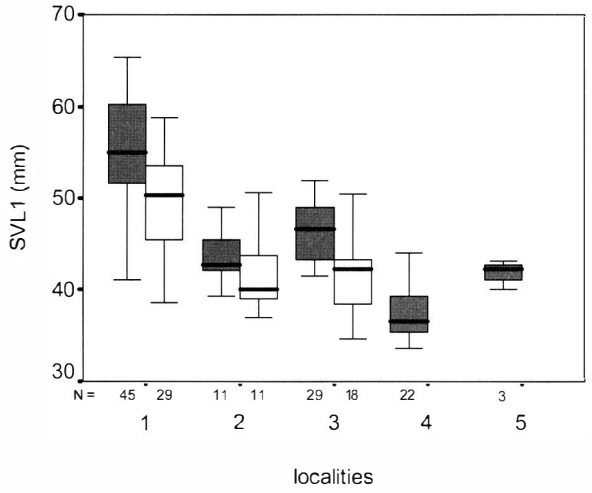

FIG. 5. Back-calculated SVL at the time of the 1st hibernation in *Lacerta agilis boemica* (shaded figures) and *L. strigata* (unshaded figures) from five localities in the eastern North Caucasus. Boxplots as in Fig. 4. Localities: 1- Kostek, 2- Sergokala, 3- Khuchni, 4- Termenlik, 5- Kuli.

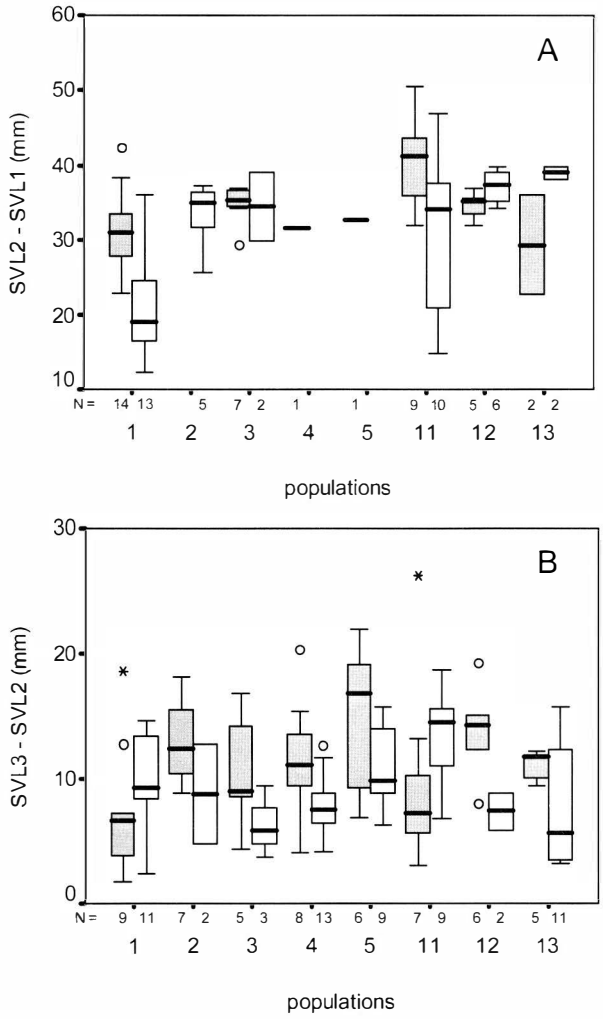

FIG 6. Individual annual increments of back-calculated SVL between the first and second hibernation (A) and between the second and third hibernation (B) in *Lacerta agilis boemica* and *L. strigata* in the eastern North Caucasus. Boxplots as in Fig. 4.

those of females in all eight populations (sign test: $P<0.01$). Sex differences were also significant in two individual populations: *L. agilis* from Sergokala and Termenlik. The largest magnitude of sex differences was found in samples of *L. agilis* from Kostek and Sergokala (Appendix 2).

On the whole, asymptotic SVL and characteristic growth rate exhibited no pronounced interpopulational differences. However, in both sexes, but especially in the females, *L. agilis* from Sergokala showed a lower asymptotic SVL than the other study populations (Appendix 2).

The 80th percentiles were in most cases rather similar to the asymptotes of the von Bertalanffy growth model (Appendix 2): in eleven of the sixteen study samples the difference between the two estimators varied from –2.1 to +1.6. In the other five samples, however, the absolute value of this difference ranged from 4.5-7.6. Like the asymptotes of the growth equation, the 80th percentiles were higher in males than in females (in seven out of the eight populations), with the largest sex differences found in *L. agilis* from Kostek and *L. strigata* from Sergokala (Appendix 2). Between populations, the 80th percentiles showed the lowest values in *L. agilis* from Sergokala as was the case for the asymptotes.

Annual increments in SVL. Back-calculating SVL from bone growth marks provided not only age-size data (Fig. 3), but also individual growth trajectories from which growth increments between consecutive hibernations can be computed. SVL increments between the first and second hibernations (SVL_2-SVL_1) which incorporated most of the growth in all study populations are summarised in Fig. 6A. In the lowland locality (Kostek) which provided appropriate sample sizes for all species/sex combinations (Fig. 6A), *L. strigata* tended to grow faster than the syntopic *L. agilis* (Mann-Whitney test, $P=0.020$ for males, $P=0.041$ for females), and the males grew faster on average than the females in both species (Mann-Whitney test, $P<0.001$ for *L.agilis*, $P=0.035$ for *L. strigata*). This pattern of differences also persisted if the effect of SVL_1 was statistically removed by use of the residuals of the linear regression of SVL_2-SVL_1 on SVL_1 (total data in Fig 6A) instead of the absolute SVL increments (Mann-Whitney test provided $P<0.05$ for all four comparisons). We employed these nonparametric tests because heterogeneity of variances in SVL_2-SVL_1 precluded use of GLM procedures.

For seven study samples, in which at least two specimens of unequal age possessed LAG1 and LAG2, Spearman rank correlation coefficient between SVL_2-SVL_1 and Age was computed. In all seven samples this correlation was negative (sign test: $P<0.05$), being significant in one sample (*L. strigata*, Kostek, females, $r_s=-0.874$, $P=0.001$).

SVL increments between the second and third hibernations (SVL_3-SVL_2) provided much larger samples than SVL_2-SVL_1 (Fig. 6B), and no heterogeneity of variances was found. ANOVA with SVL_3-SVL_2 as the dependent variable, and Sex, Locality, and Species as

the factors showed significant effects of Sex ($F_{1,76}$= 7.435, P=0.008) and Sex × Locality interaction ($F_{4,76}$= 3.733, P=0.007). All other factors and interaction terms were non-significant. In most populations the males tended to have higher growth increments than the females, but both species exhibited the opposite pattern in Kostek (Fig. 6B). However, just in Kostek, males grew faster than the females between the first and second hibernations (Fig. 6A).

An ANCOVA with SVL_3-SVL_2 as the dependent variable, SVL_2 as the covariate, and Sex, Locality and Species as the factors showed significant effects of the Covariate ($F_{1,76}$=71.10, P<0.001) and Sex ($F_{1,76}$=14.38, P=0.001) while all other sources were non-significant or marginally significant (P=0.036-0.069 for Locality and Species, P=0.138-0.612 for the interaction terms). Thus, the variation in SVL_2 cannot be solely responsible for the observed sex differences in body growth between the second and third hibernations. However, this variation might explain the deviating pattern of sex differences for SVL_3-SVL_2 in Kostek, because after correcting for SVL_2 (ANCOVA) Sex × Locality interaction was not any more significant.

DISCUSSION

AGE STRUCTURE

We believe the age structures of our study samples are, in general, representative for the whole populations. When specimens were collected, efforts were made to catch every individual of both species on the study sites until an appropriate sample size (25-30 specimens of each sex) had been achieved. Although some differential, age-related catchability cannot be excluded, we see no reason to suspect a substantial sampling bias, at least for adult animals.

In our lowland and submontane localities the age structure of both study species is characterised by a maximum age of 4-6 years and predominance of 2- and 3-yr-olds among adult individuals (Fig. 2). Similar age compositions were reported for several *L. agilis* populations in West Ukraine and South Siberia (reviewed in: Roitberg & Smirina, 1995) and most other medium-sized and smaller species of lacertid lizards studied so far (e.g. Orlova & Smirina, 1983; Smirina *et al.*, 1984; Castanet, 1994).

In contrast, in the mountain populations of *L. a. boemica* the maximum ages were 7-8 years, with 2- and 3-yr-old animals constituting some 50% of adults (Fig. 2). Comparable age structures were found in two North European *L. agilis* populations (Strijbosch & Creemers, 1988; Olsson & Shine, 1996).

The increase in longevity and mean age in the mountain populations as compared to their conspecifics from lower elevations (Fig. 2) has already been reported for various lizard species (Ballinger, 1979; Melkumyan, 1983; Roitberg & Smirina, 1995; Rohr, 1997; Wapstra *et al.*, 2001) and many amphibians (reviews in: Smirina, 1994; Miaud *et al.*, 2000). Like some other altitudinal trends considered above, this pattern can be related to the length of activity season: shorter activity in cooler climate should reduce the risk of predation or other accidental death (Adolph and Porter, 1993; Ryser, 1996; Willemsen & Hailey, 2001). Additionally, Ballinger (1979) suggested that there could be lower predation pressure due to a reduced number of predator species in areas with colder climates which could explain the enhanced longevity in highland lizard populations. Although this may be the case for our study populations (the snake *Coluber caspius*, an effective predator of green lizards, does not occur in the two mountain sites, while it is rather common at lower elevations), the altitudinal differences in predator diversity are not as evident in our study as the differences in length of activity season.

Some features of interlocality variation revealed in this study differed from our expectations; (1) The lowland *L. agilis* population showed higher mean adult age than the submontane populations; (2) Although the altitudinal differences between Termenlik and the two submontane localities, Sergokala and Khuchni, are much lower than those between Termenlik and Kuli (Appendix 1), the *L. agilis* from Termenlik exhibited the same (if not a higher) mean age and longevity as their high-mountain conspecifics in Kuli (Fig. 2). We have no hypothesis to explain pattern 1; the pattern is minor in any case when compared to the differences between lowland and submontane populations on one side and mountain populations on the other. Pattern 2 can be related to certain climatic characteristics of Termenlik (precipitation, evaporation, number of days with mean air temperature above 10°C – Appendix 1) which would make the overall time of lizard activity comparable to that of the highlands. It seems, it is the length of activity season that determines the main trend under discussion, and populations living in colder environments would exhibit higher mean age and longevity irrespective of altitude (e.g., Saint Girons *et al.*, 1989). Similarly, a *Timon lepidus* population from a hot region, where the annual activity was reduced by aestivation, showed higher mean age than a population living in a cooler and mesic environment (Mateo & Castanet, 1994).

PATTERN OF LAG RESORPTION AND POSSIBLE BIAS IN BACK-CALCULATED DATA

Partial or complete loss of LAG1 due to endosteal resorption generally did not disturb age determination as there was only a small overlap between the distribution of marrow cavity diameters and the distribution of D_1 in the study samples. However, this resorption is a serious problem for growth inference from skeletochronological data as it precludes measuring the corresponding ring diameters (D_1) and back-calculating the corresponding SVLs. A high resorption frequency not only reduces the amount of data, but may also bias our estimations of mean values of ring diameters. For merely mechanical reasons, growth rings with smaller diameters are expect-

TABLE 3. LAG1 diameter in different age-sex groups within populations. Only the Population/Sex combinations with $n>1$ for both age groups were considered. Diff=Mean of Adults - Mean of Yearlings.

Populations (as in Fig. 2)	Sex	Yearlings (Age=1+)			Adults (Age >1+)			Diff.
		Mean	SD	n	Mean	SD	n	
1	m	0.667	0.017	5	0.611	0.061	14	-0.056
	f	0.588	0.058	12	0.574	0.077	14	-0.014
2	f	0.442	0.022	2	0.451	0.028	7	0.009
3	m	0.531	0.038	9	0.535	0.045	7	0.004
	f	0.528	0.028	11	0.516	0.066	2	-0.012
11	m	0.561	0.052	7	0.545	0.084	9	-0.016
	f	0.518	0.087	3	0.531	0.067	10	0.013
13	m	0.460	0.035	6	0.527	0.088	2	0.067
	f	0.459	0.016	8	0.379	0.002	2	-0.080

ed to be destroyed more frequently than larger rings. Thus, the mean D_1 (and body length at the first hibernation) might be slightly overestimated (*cf.* Ryser, 1996). Such a bias in the size distribution of a growth mark might be called pseudoselection because it is obviously unrelated to size-dependent survival (true natural selection), but under a high resorption frequency it may imitate true natural selection with a higher survival rate for larger juveniles. The latter was reported for two frog species in which the mean D_1 of adults was higher than that of young animals under a low resorption frequency (Esteban *et al.*, 1999; Esteban & Sanchiz, 2000). The pronounced negative correlation between the resorption frequency and mean diameter of D_1, found for the variation between populations (Table 2), seems to be in line with our pseudoselection hypothesis. At the same time, no consistent trend was revealed in comparisons of the mean D_1 between adults and yearlings within populations (Table 3). Possibly, the proposed pseudoselection can be compensated by other factors which affect the rate of endosteal resorption.

Evaluation of the Back-calculated Data

Virtually all body growth data in this study were obtained with the back-calculation technique which had not previously been applied to squamate reptiles. Although no other data on SVL growth were available for our study populations, some indirect validation of back-calculated SVL values can be made. For both species, back-calculated SVLs at hatching (this study) were located within the range of values obtained in captivity (Langerwerf, 1980; Roitberg, 1989; Warnecke, 2000). This correspondence is very important because the highest errors of back-calculation are expected just for lowest values of D_i and SVL_i (Marunouchi *et al.*, 2000).

For SVL at the end of the first and subsequent growth seasons, we compared back-calculated and field data by maximum values (in fact we used the two largest values to reduce variation by chance; we did not use upper percentiles here because it was difficult to appropriately delimit the sample for our field data). Maximum values should be less sensitive to pseudoselection and to other sources of sampling bias (e.g. a proportion of individuals whose growth rate or activity was reduced due to temporal or local effects) than the means or medians. As SVL distributions were effectively continuous, maximum SVLs at specific ages are expected to manifest the body growth potential of a given population in a particular environment and should therefore be biologically meaningful.

The largest values of back-calculated SVL at the first hibernation recorded in our lowland site (Kostek) were 64-65 mm in *L. agilis* and 58-59 mm in *L. strigata*. In large juvenile samples caught in September-October 1981-1984 in another lowland locality (Makhachkala, ca. 100 km SE from Kostek, a mark-recapture study) the largest values were 61-63 mm for *L. agilis* and 54-55 mm for *L. strigata* (Roitberg, 1989).

The largest back-calculated SVL at the second hibernation in the lowland locality was 94-95 mm in *L. agilis* and 92-93 mm in *L. strigata*. In Makhachkala, some *L. agilis* yearlings could reach 87-90 mm in August – September of their 2nd activity season (E. S. Roitberg, unpublished data), and one yearling *L. strigata* from Kostek had a SVL of 92 mm on 7 Sept. 1984.

Even though a slight overestimation of SVL for the young ages might be suspected, the overall conformity between back-calculated and comparable field data considered above suggests the former to be suitable at least for rough estimations. Additional support for the plausibility of our back-calculated data can be seen in the validation study on *Rana japonica* (Marunouchi *et al.*, 2000, see our Methods section). At least, the correlation between body length and bone thickness in our study samples was as high as that in *R. japonica*, and the femur growth is expected to be even more integrated in the body growth than that of a phalanx. However, validation studies on lizards are necessary to reliably estimate the

mean and range of possible deviations of our back-calculated data from actual values.

Sergeev (1937) used the Dahl-Lea formula for back-calculating age-specific body lengths from carapace growth rings in several turtle species. Like our study, he reported conformity of back-calculated data with direct measurements on young individuals of known age (but see Wilson *et al.*, 2003 for concerns about use of carapace growth rings as recording structures).

Patterns of Variation in Age-specific SVLs and Annual SVL Increments

The back-calculation procedure provides estimations of individual body size at ages before capture, thus multiplying the total amount of age-size data, especially for older animals. It estimates the body size at specific ages, just at the end of a growth season (or at the beginning of the next one), providing comparable data from samples collected at different times of the season.

The decrease of mean and maximum body length at the 1st hibernation (SVL_1) with altitude can be explained by the differences in the time available for growth. In the mountains, juveniles hatch later and go into hibernation earlier than those in low-elevation sites. Lower mean SVL_1 in *L. strigata* as compared to the syntopic *L. agilis* can be explained in the same way: *L. strigata* hatched later in the season (Roitberg, 1989; E. S. Roitberg, unpublished data) due to a longer incubation time (Zakharov *et al.*, 1982).

In the lowland populations of both species, the largest juveniles enter their first hibernation and, as yearlings, start their second activity season with a SVL of 60-65 mm (Fig. 3, Fig. 5). Minimal SVL at the first reproduction in lowland Daghestan was estimated as 70 mm (Khonyakina, 1970; see also Tertyshnikov, 2002 for an adjacent region). In our lowland site, about 50% of yearling *L. agilis* and a few yearling *L. strigata* captured in the first half of June were 70-77 mm in SVL. Five of them (four female *L. agilis* and one female *L. strigata*) had oviductal eggs so in lowland Daghestan, some yearling females of the study species do reproduce. This finding is noteworthy because even for warm climate regions an age of maturity at 22-23 months was previously reported for both study species (Muskhelishvili, 1970; Khonyakina, 1970, 1972; Darevskij, 1984; Tertyshnikov, 2002), except *L. strigata* from south-western Turkmenistan for which the reproduction of yearlings was reported (Shammakov, 1981). No yearling females with oviductal eggs were found in the study samples from the submontane and mountain sites (E. S. Roitberg, unpublished data). We believe that the proportion of reproducing female yearlings may be substantial in our lowland locality (at least in *L. agilis*), it is rather small in our submontane localities, and no yearlings reproduce in the mountain sites. A similar shift in age of maturity in colder climates has been shown for many lizard species (Ballinger, 1979; Heulin, 1985; Rohr, 1997; Wapstra *et al.*, 2001; but see Grant & Dunham, 1990 for an opposite shift in age at maturity at high elevation).

The proposed altitudinal differences in the mean age at first reproduction in the study species agree with the model of Adolph & Porter (1996) which views variation in age and size at maturity in lizards as a product of the proximate effect of temperature on their growth and maturation pattern. Individuals living in warmer areas are predicted to mature earlier (at a relatively small size), whereas their conspecifics from cool environments delay maturity (and thus invest available energy to further growth) because they cannot reach an appropriate size by the time at which reproduction is still suitable. This proposal might also explain some patterns of variation in age-specific SVL and annual growth increments revealed in this study if we assume that the energetic costs of reproduction are considerably higher in females than in males (Nagy, 1983; Anderson & Vitt, 1990).

In the lowland locality, males grew rapidly between the first and second hibernations in both species (Fig. 6A). By their third activity season, many of the males become quite large (some individuals of both species can exceed 90 mm in SVL that corresponds to some 90% of their final size – Fig. 4A), and their further growth slows down (Fig. 6B) as they approach their final (asymptotic) body length. In the hot and dry climate of the Ararat valley in Armenia, *L. strigata* can also approach final SVL at the time of entering the 2nd hibernation (Melkumyan, 1983). Lowland females exhibited much lower SVL increments in their second activity season than the males, particularly in *L. agilis* (Fig. 4A, Fig. 6A), probably because a substantial proportion of them did reproduce and allocated plenty of energy to egg production at the expense of body growth. At higher elevations, the study species exhibited no sex differences in body growth between the first and second hibernations (Fig. 4A, Fig. 6A), but during the following season the females grew less intensively than the males (Fig. 6B). It seems, the phase of lower body growth in females relative to males, being associated with the first reproduction, is merely shifted in the lowland locality to younger ages, because the warmer climate (Appendix 1) accelerates growth and maturation (Adolph & Porter, 1996). A lower growth rate of young reproducing females relative to males of the same age was reported for various lizard species (e.g., Anderson & Vitt, 1990; Howland, 1992; Rocha, 1995).

Asymptotic Size

For species with asymptotic growth after maturity, asymptotic size is affected by a much shorter list of proximate factors than average size (Stamps, 1993). Therefore, for comparative studies focusing on differences among populations or between sexes, asymptotic size is preferable to mean adult size (Stamps & Andrews, 1992; Stamps, 1993; Brown *et al.*, 1999).

There are some problems with estimating the mean asymptotic size from growth equations using nonlinear regression. Like the slope and intercept of a linear regression, the parameters A (asymptote) and k (characteristic growth rate) of a growth equation are es-

timated simultaneously. Their statistical estimates are thus not independent from one another and any factor that affect estimates of k can also influence estimates of A (Stamps *et al.*, 1994). Therefore, a deficiency of old, full-grown individuals or a strong variation in the proportion of young, small animals in a particular sample might bias the considered estimates (James, 1991; Stamps *et al.*, 1994). This is a probable explanation for the extremely high k and low A in female *L. agilis* from Sergokala (Appendix 2) because this sample included many data points for age 1 while only one data point for ages higher than 2 (Fig. 3).

Furthermore, through the use of group data (a composite of points of individual growth curves) to model individual growth, the resulting growth curve can be biased by differential survival of fast-growing and slower-growing individuals (Mina & Klevezal, 1976; Bruce *et al.*, 2002). The latter phenomenon, in the form of a lower survival of fast-growers, has been reported for a range of lizard species (Smirina & Tsellarius 1996; Sorci *et al.* 1996; Olsson & Shine, 2002). The negative correlation between the individual age at capture and the SVL increment between the first and second hibernations revealed in the present study may similarly indicate a lower survival of fast-growing animals as compared to those with lower growth rate. If this bias is moderate in magnitude or, at least, does not differ substantially between populations, cautious use of growth curves for comparative purposes seems suitable.

The 80th percentile suggested as an alternative estimator of mean asymptotic SVL (Brown *et al.*, 1999) is likely to be resistant to any variation outside the upper area of character distribution; it is also not sensitive to outliers. However, if the proportion of full-grown individuals is very low (due to a sampling bias or a low survival of older animals) this estimate may be even more biased than the estimate of A from the growth equation.

We believe that if the two estimators of asymptotic size, which are unrelated statistically and calculated from different sets of data (back-calculated SVLs for the growth-based estimator and SVLs at capture for the percentile), provide comparable values and similar patterns of variation, these are likely to be biologically meaningful. Viewing our results (Appendix 2) in this way we can state that in the study populations, males tend to have larger final size than females, but the extent of the differences varied between populations, being rather large (11-12 mm for the different estimators) in *L. agilis* from Kostek and quite small (0-3 mm) in *L. agilis* from Kuli. The only clear pattern of the interpopulational differences is a lower final size of *L. agilis* from Sergokala. Apart from a true geographic variation in adult SVL (e.g., Saint Girons *et al.*, 1989; Mateo & Castanet, 1994), this pattern might also result from an interannual fluctuation in age structure or growth rate because the whole study sample was collected during a few days of a single season (Table 1).

Sexual Size Dimorphism (SSD)

A pronounced male-biased SSD found for adult *L. agilis* SVL in a few study sites is noteworthy because virtually all other *L. agilis* populations studied thus far exhibited either a female-biased SSD or no obvious sex differences for SVL (see e.g. Olsson & Shine, 1996; Amat *et al.*, 2000 for West Europe; see Darevsky *et al.*, 1976 for more eastern parts of the species range).

Modern theory views SSD as a complex phenomenon, an outcome of several interacting factors (e.g. Shine, 1990; Stamps, 1993; Braña, 1996; Cox *et al.*, 2003). The most prevalent explanation for male-biased SSD in reptiles is intrasexual selection which favours larger males as they are generally more successful in male combat (e.g. Anderson & Vitt, 1990; Cox *et al.*, 2003). We can hypothesise that in *L. agilis* the intensity of this selection differs between subspecies or geographic regions (e.g. due to some differences in territoriality or other aspects of social behaviour; *cf.*: Shine & Fitzgerald, 1995) resulting in adaptive divergence in the SSD pattern. As was recently shown, intraspecific SSD variation in lizards can have an evolutionary component (Zamudio, 1998). A pronounced morphological (Roitberg, 1987) and genetic (Kalyabina *et al.*, 2001) separation of *L. a. boemica* from the rest of the species could argue for a phylogenetic determination of the distinctive male-biased SSD. However, phylogenetic determination can hardly explain the pronounced microgeographic variation of SSD pattern within *L. a. boemica*.

Apart from selective pressures, SSD may be influenced by proximate factors and reflect local, environmentally-induced variation in growth, maturation and survival rates (Shine, 1990; Stamps, 1993; Watkins 1996). This is especially true for reptiles and other animals whose body growth continues asymptotically after sexual maturity (Stamps, 1993). Taking into account that the different estimators of adult body length generally showed the strongest SSD in the lowland *L. agilis* population, we believe microgeographic variation for SSD within *L. a. boemica* is largely determined by proximate factors related to the length of activity season. According to theoretical predictions (Adolph & Porter, 1996; see above) and data on altitudinal variation of various reptile species (e.g. Ballinger, 1979; Rohr, 1997; Wapstra *et al.*, 2001), lizards from the lowland locality are expected to have a smaller average size at maturity than their conspecifics from higher elevations. In many reptiles the size at maturity was shown to strongly correlate with the final size (Andrews, 1982; Shine, 1990; Stamps *et al.*, 1998). As reproduction is expected to more strongly inhibit body growth in females than in males, earlier maturation might be responsible for smaller female body length and the consequently male-biased SSD in the lowland populations. An additional reason for smaller female size in warmer climates may be higher annual reproductive expenditures because in such an environment many females

produce two clutches per season (Baranov *et al.*, 1976; Khonyakina & Ferkhatova, 1977). These hypotheses should be tested through further research to better evaluate proximate and evolutionary causes for geographic variation of SSD in *L. agilis*.

Comparisons Between the Study Species

L. a. boemica and *L. strigata*, two closely related species occupying the same environment, were compared for several age-specific SVLs and annual growth increments, asymptotic SVL, and age composition. Compared with *L. a. boemica*, *L. strigata* is characterised by longer egg incubation and later hatching time. Consequently, before and soon after their first hibernation, juvenile *L. strigata* are on average smaller than juveniles of the syntopic *L. a. boemica* (Fig. 5). However, at least in the lowland site, yearling *L. strigata* grew faster than those of *L. a. boemica* (Fig. 6A), so no substantial differences in SVL between the study taxa could be detected after the second hibernation (Fig. 4). These differences conform to the pattern of spatial distribution of the study species in the eastern North Caucasus: *L. strigata* occupies a much larger range of lowland localities than *L. a. boemica*, but the latter expands substantially further in the mountains (Roitberg *et al.*, 2000).

Unlike other life-history studies comparing two related syntopic lizard species (Tinkle & Dunham, 1986; Strijbosch & Creemers, 1988) we revealed no differences in age composition. This might be attributed to closer relatedness between our study taxa which are not merely congeneric but belong to the same subgenus (Arnold, 1989). Another probably even more relevant difference from the above investigations is that our study taxa have similar adult sizes. Adult size was shown to be the major determinant of variation for the other life-history and demography traits among related lizard species (Dunham *et al.*, 1988; Bauwens & Diaz-Uriarte, 1997; Molina-Borja & Rodríguez-Domínguez, 2004).

Final Remarks

Much of the variation for age structure, body growth and sexual size dimorphism revealed in *L. a. boemica* and *L. strigata* along an altitudinal gradient in the eastern North Caucasus can be related to the length of activity season in line with the models of Adolph & Porter (1993, 1996). These models offer proximate explanations for the trends of life-history variation along environmental gradients that were previously considered as unequivocal adaptations (e.g., Ballinger, 1983; Dunham *et al.*, 1988). Although several experimental studies revealed a genetic (population-specific) component of the interpopulational variation for growth rates in lizards (Ballinger, 1979; Ferguson & Brockman, 1980; Ferguson & Talent, 1993; Niewiearowski & Roosenburg, 1993), some recent studies with a powerful design could identify only the environmental sources of such variation (Sorci *et al.*, 1996; Qualls & Shine, 2000; Lorenzon *et al.*, 2001). The latter was therefore treated as phenotypic plasticity within the reaction norm (Gotthard & Nylin, 1995). Obviously, not only the patterns of intraspecific life-history variation in lizards are diversified but also the extent to which they are genetically fixed. The progress in our understanding this diversity is primarily determined by intensive mark-recapture and experimental studies of model species. However, such studies can cover only a small portion of taxonomical and ecological diversity of situations which deserve to be investigated. Skeletochronology can substantially extend the range of the study populations because valuable data on individual age and growth can be obtained during a relatively short time. Our report has shown that much ecologically relevant data, which would otherwise require a long-term field study, can be obtained from museum samples if they are large enough and appropriately collected (the amount of data could be even greater if the resorption frequency of younger LAGs had not been so high). The back-calculation method proved to be a promising tool to extract quantitative data on lizard body growth from skeletochronological records.

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REFERENCES

Adolph, S. C. & Porter, W. P. (1993). Temperature, activity, and lizards life histories. *American Naturalist* **142**, 273-295.

Adolph, S. C. & Porter, W. P. (1996). Growth, seasonality, and lizards life histories. *Oikos* **77**, 267-278.

Amat, F., Llorente, G. A. & Carretero, M. A. (2000). Reproductive cycle of the sand lizard (*Lacerta agilis*) in its southwestern range. *Amphibia-Reptilia* **21**, 463-476.

Anderson, R. A. & Vitt, L. J. (1990). Sexual selection versus alternative causes of sexual dimorphism in teiid lizards. *Oecologia* **84**, 145-157.

Andrews, R. M. (1982). Patterns of growth in reptiles. In *Biology of the Reptilia. Vol. 13*, 273-320. Gans, C. & Pough, F. H. (Eds.) London: Academic Press.

Arakelyan, M. S. (2002). The study of age, growth, and longevity in the triploid hybrids of rock lizards of the genus *Darevskia* in Armenia. *Russian Journal of Herpetology* **9**, 63-68.

Arnold, E. N. (1989). Towards a phylogeny and biogeography of the Lacertidae: relationships within

an Old-World family of lizards derived from morphology. *Bulletin of the British Museum (Natural History)* **25**, 203-257.

Ballinger, R. E. (1979). Intraspecific variation in demography and life history of the lizard, *Sceloporus jarrovi*, along an altitudinal gradient in southeastern Arizona. *Ecology* **60**, 901-909.

Ballinger, R. E. (1983). Life-history variations. In *Lizard ecology: studies of a model organism*, 241-260. Huey, R. B., Pianka, E. R. & Schoener, T. W. (Eds.) Cambridge, Massachusetts: Harvard University Press.

Baranov, A. S., Strel'tsov, A. B. & Tertyshnikov, M. F. (1976). Reproduction. In *Prytkaya yashcheritsa. Monographicheskoye opisaniye vida*, 214-226. /The sand lizard: A monographic description of the species. Yablokov, A. V. (Ed.). Moscow: Nauka [in Russian].

Bauwens, D. & Diaz-Uriarte, R. (1997). Covariation of life-history traits in lacertid lizards: a comparative study. *American Naturalist* **149**, 91-111.

Bischoff, W. (1988). Zur Verbreitung und Systematik der Zauneidechse, *Lacerta agilis* Linnaeus 1758. *Mertensiella* **1**, 11-30.

Braña, F. (1996). Sexual dimorphism in lacertid lizards: male head increase vs female abdomen increase? *Oikos* **75**, 511-523.

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

Bruce, R., Castanet, J., & Francillon-Vieillot, H. (2002). Skeletochronological analysis of variation in age structure, body size, and life history in three species of desmognathine salamanders. *Herpetologica* **58**, 181-193.

Castanet, J. (1994). Age estimation and longevity in Reptiles. *Gerontology* **40**, 174-192.

Castanet, J. & Smirina, E. M. (1990). Introduction to the skeletochronological method in amphibians and reptiles. *Annales des Sciences Naturalles Zoologie, Paris 13e Serie* **11**, 191-196.

Castanet, J. & Baez, M. (1991). Adaptation and evolution in *Gallotia* lizards from the Canary Islands: age, growth, maturity and longevity. *Amphibia-Reptilia* **12**, 81-102.

Castanet, J., Meunier, F. & Ricqlès, A. (1977). L'enregistrement de la croissance cyclique par le tissu osseux chez les vertébrés poïkilothermes: données comparatives et essai de synthèse. *Bulletin Biologique de la France et de la Belgique* **111**, 183-202.

Castanet, J., Francillon-Vieillot, H., Meunier, F.J. & de Ricqles, A. (1993). Bone and individual aging. *Bone. Vol. 7, Bone growth – B*, 245-283. Hall, B.K. (Ed.) Boca Raton: CRC.

Cox, R., Skelly, S. & John-Alder, H. B. (2003). A comparative test of adaptive hypotheses for sexual size dimorphism in lizards. *Evolution* **57**, 1653-1669.

Darevskij, I. S. (1984). *Lacerta strigata* Eichwald 1831 - Kaspische Smaragdeidechse. *Handbuch der Reptilien und Amphibien Europas, Band 2/1, Echsen II.* (Ed Böhme W.) pp. 82-99. (AULA - Verlag: Wiesbaden.)

Darevsky, I. S., Shcherbak, N. N. & Peters, G. (1976). *Systematics and intraspecific structure*, 53-95. In *Prytkaya yashcheritsa. Monographicheskoye opisaniye vida* /The sand lizard: A monographic description of the species/. Yablokov A.V. (Ed.). Moscow: Nauka. [in Russian].

Dunham, A. E., Miles, D. B. & Reznik, D. N. (1988). Life history patterns in squamate reptiles. In *Biology of the Reptilia. Vol. 16*, 441-522. Gans, C. & Pough, F. H. (Eds.) London: Academic Press.

Esteban, M. & Sanchiz, B. (2000). Differential growth and longevity in low and high altitude *Rana iberica* (Anura, Ranidae). *Herpetological Journal* **10**, 19-26.

Esteban, M., Garcia-Paris, M., Buckley, D. & Castanet, J. (1999). Bone growth and age in *Rana saharica*, a water frog living in a desert environment. *Annali Zooogici Fennici* **36**, 53-62.

Ferguson, G. W. & Brockman, T. (1980). Geographic differences of growth rate of *Sceloporus* lizards (Sauria: Iguanidae). *Copeia* **1980**, 259-264.

Ferguson, G. W. & Talent, L. G. (1993). Life history traits of the lizards *Sceloporus undulatus* from two populations raised in a common laboratory environment. *Oecologia* **93**, 88-94.

Francis, R. I. C. C. (1990). Back-calculation of fish length: a critical review. *Journal of Fish Biology* **36**, 883-902.

Gotthard, K. & Nylin, S. (1995). Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. *Oikos* **74**, 3-17.

Grant, B. W. & Dunham, A. E. (1990). Elevational covariation in environmental constraints and life histories of the desert lizard *Sceloporus merriami*. *Ecology* **71**, 1765-1776.

Heulin, B. (1985). Maturité sexuelle et age a la premiere reproduction dans une population de plaine de *Lacerta vivipara*. *Canadian Journal of Zoology* **63**, 1773-1777.

Howland, J. (1992). Life history of *Cophosaurus texanus*: environmental correlates and interpopulational variation. *Copeia*: 82-93.

James, C. D. (1991). Growth rates and ages at maturity of sympatric scincid lizards (*Ctenotus*) in Central Australia. *Journal of Herpetology* **25**, 284-295.

Kalyabina, S. A., Milto, K. D., Ananjeva, N. B., Legal, L., Joger, U. & Wink, M. (2001). Phylogeography and systematics of *Lacerta agilis* based on mitochondrial cytochrome *b* gene sequences: first results. *Russian Journal of Herpetology* **8**, 149-158.

Khonyakina, Z. P. (1970). Data on biology of *Lacerta agilis* in Daghestan. In *Issledovaniya po zoologii i parazitologii v Daghestane*, 88-93. Makhachkala: DGU [in Russian].

Khonyakina, Z. P. (1972). *Lacerta strigata* and *L. media* of Daghestan. In *Issledovaniya po zoologii i parazitologii v Daghestane*, 150-159. Makhachkala: DGU [in Russian].

Khonyakina, Z. P. & Ferkhatova, Z. A. (1977). Characteristics of populations of *Lacerta strigata* from a lowland and a submontane locality of Daghestan. In *Voprosy gerpetologii*, 216-217. Leningrad. [in Russian].

Kratochvil, L. & Frynta, D. (2002). Body size, male combat and the evolution of sexual dimorphism in eublepharid geckos (Squamata: Eublepharidae). *Biological Journal of Linnean Society* **76**, 303-314.

Langerwerf, B. (1980). The Caucasian green lizard, *Lacerta strigata*, Eichwald 1831, with notes on its reproduction in captivity: *British Herpetological Society Bulletin* **1**, 23-26.

Lorenzon, P., Clobert, J. & Massot, M. (2001). The contribution of phenotypic plasticity to adaptation in *Lacerta vivipara*. *Evolution* **55**, 392-404.

Marunouchi, J., Kusano, T. & Ueda, H. (2000). Validity of back-calculation methods of body size from phalangeal bones: an assessment using data for *Rana japonica*. *Current Herpetology* **19**, 81-89.

Mateo, J. A. & Castanet, J. (1994). Reproductive strategies in three Spanish populations of the ocellated lizard, *Lacerta lepida* (Sauria, Lacertidae). *Acta Oecologica* **15**, 215-229.

Melkumyan, L. S. (1983). The growth of *Lacerta strigata* in lowland and mountains. *Zoologichesky Zhurnal* **62**, 580-584.

Miaud, C., Guyetant, R. & Faber, H. (2000). Age, size, and growth of the alpine newt, *Triturus alpestris* (Urodela: Salamandridae), at high altitude and a review of life-history trait variation throughout its range. *Herpetologica* **56**, 135-144.

Mina, M. V. & Klevezal, G. A. (1970). 'The principles of investigation of recording structures'. *Uspekhy Sovremennoi Biologii* **7**, 341-352. [in Russian].

Mina, M. V. & Klevezal, G. A. (1976). '*Growth of animals*'. Moscow: Nauka, 291 p. [in Russian].

Molina-Borja, M. & Rodríguez-Domínguez, M. A (2004). Evolution of biometric and life-history traits in lizards (*Gallotia*) from the Canary Islands. *Journal of Zoological Systematics and Evolutionary Research* **42**, 44-53.

Muskhelishvili, T. A. (1970). *Reptiles of East Georgia*, Tbilisi: Metsniereba. [in Russian].

Nagy, K. A. (1983). Ecological energetics. In *Lizard ecology: studies of a model organism*, 24-54. Huey, R.B., Pianka, E.R. & Schoener, T.W. (Eds.). Cambridge, Massachusetts: Harvard University Press.

Niewiearowski, P. & Roosenburg, W. M. (1993). Reciprocal transplant experiments reveals sources of variation in growth rates of the lizard *Sceloporus undulatus*. *Ecology* **74**, 1992-2002.

Olsson, M. & Shine, R. (1996). Does reproductive success increase with age or with size in species with indeterminate growth - a case study using sand lizards (*Lacerta agilis*). *Oecologia* **105**, 175-178.

Olsson, M. & Shine, R. (2002). Growth to death in lizards. *Evolution* **56**, 1867-1870.

Orlova, V. F. & Smirina, E. M. (1983). Age determination of the *Lacerta derjugini* in natural populations. *Biologicheskie Nauki* **9**, 53-57 [in Russian].

Pianka, E. R. & Vitt, L. J. (2003). *Lizards: windows to the evolution of biodiversity*. Berkeley: (University of California Press.

Qualls, F. J. & Shine, R. (2000). Post-hatching environment contributes greatly to phenotypic variation between two populations of the Australian garden skink, *Lampropholis guichenoti*. *Biological Journal of Linnean Society* **71**, 315-341.

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

Ricker, W. E. (1973). Linear regressions in fishery research. *Journal of Fishery Research Board Canada* **30**, 409-434.

Rocha, C. F. D. (1995). Growth of the tropical sand lizard *Liolaemus lutzae* in Southeastern Brazil. *Amphibia-Reptilia* **16**, 257-264.

Rohr, D. H. (1997). Demographic and life-history variation in two proximate populations of a viviparous skink separated by a steep altitudinal gradient. *Journal of Animal Ecoogy* **66**, 567-578.

Roitberg [Roytberg], E. S. (1982). Evaluation of the possibility of hybridization between *Lacerta agilis* and *L. strigata* (Sauria, Lacertidae) in Daghestan. *Zooogichesky Zhurnal* **61**, 249-253.

Roitberg [Roytberg], E. S. (1987). Discrete variants of scutellation in the lizards *Lacerta agilis* and *L. strigata* from Daghestan. *Proceedings of the Zoological Institute*, Leningrad **158**, 131-138.

Roitberg, E. S. (1989). Variation of scalation characters in *Lacerta agilis* L. and *L. strigata* Eichw. in an area of their sympatry. PhD. Thesis, Daghestan Univ., Makhachkala, 256 p. [in Russian].

Roitberg [Roytberg], E. S. & Smirina E. M. (1995). Age- and size composition of some populations of *Lacerta agilis boemica* and *L. strigata* (Sauria, Lacertidae) from the eastern North Caucasus. In *Scientia Herpetologica*, 224-228. Llorente G.A., Carretero M.A. & Santos X. (Eds.). Barcelona: Societas Europaea Herpetologica.

Roitberg, E. S. & Smirina, E. M. (2005). The relationship between body length and femur bone thickness in *Lacerta agilis boemica* and *L. strigata*. Implications for growth inferences from skeletochronological data. In: Herpetologia Petropolitana. Ananjeva N. & Tsinenko O. (eds.), pp. 298-300. St. Petersburg, Societas Europaea Herpetologica.

Roitberg, E. S., Mazanaeva, L. F., Ilyina, E. V. & Orlova, V. F. (2000). Die Echsen Dagestans (Nordkaukasus, Russland): Artenliste und aktuelle Verbreitungsdaten (Reptilia: Sauria: Gekkonidae, Agamidae, Anguidae, Scincidae et Lacertidae). *Faunistische Abhandlungen, Staatliches Museum für Tierkunde, Dresden*, **22**, 97-118.

Ryser, J. (1996). Comparative life histories of a low- and high-elevation population of the common frog *Rana temporaria*. *Amphibia-Reptilia* **17**, 183-195.

Saint Girons, H., Castanet, J., Bradshaw, S. D. & Baron, J. P. (1989). Demographie comparee de deux populations de *Lacerta viridis* (Laurenti, 1768). *Terre et Vie* **44**, 361-386.

Schoener, T. W. & Schoener, A. (1978). Estimating and interpreting body-size growth in some *Anolis* lizards. *Copeia* **1978**, 390-405.

Sergeev, A. M. (1937). Some materials to the problem of the reptile postembryonic growth. *Zoologichesky Zhurnal*. **16**, 723-735.

Shammakov, S. M. (1981). '*Reptiles of the plain Turkmenistan*' Ashgabad: Ylym. [in Russian].

Shine, R. (1990). Proximate determinants of sexual size differences in adult body size. *American Naturalist* **135**, 278-283.

Shine, R. & Fitzgerald, M. (1995). Variation in mating systems and sexual size dimorphism between populations of the Australian python *Morelia spilota* (Serpentes: Pythonidae). *Oecologia* **103**, 490-498.

Sinsch, U., Martino, A. & Di Tada, E. (2002). Longevity and sexual size dimorphism of the Pampa de Achala copper lizard *Pristidactilus achalensis* (Gallardo, 1964). *Amphibia-Reptilia* **23**, 177-190.

Smirina, E. M. (1972). Annual layers in bones of *Rana temporaria*. *Zoologichesky Zhurnal* **51**, 1529-1534.

Smirina, E. M. (1983). Age determination and retrospective body size evaluation in live common toads (*Bufo bufo*). *Zoologichesky Zhurnal* **62**, 437-444.

Smirina, E. M. (1994). Age determination and longevity in amphibians. *Gerontology* **40**, 133-146.

Smirina, E. M. & Tsellarius, A. Yu. (1996). Aging, longevity, and growth of the desert monitor (*Varanus griseus*). *Russian Journal of Herpetology* **3**, 130-142.

Smirina, E. M., Makarov A.N. & Orlova V.F. (1984). On the age determination and some peculiarities of growth of *Lacerta praticola* in the Caucasus. In *Vid i ego produktivnost' v areale. V. Problemy gerpetologii*, 36-37. *[The species and its productivity within its range. Part. V. Problems of herpetology*. Sverdlovsk. [in Russian].

Sokal, R. R. & Rohlf, F. J. (1995). *Biometry*. W. N. Freeman: New York.

Sorci, G., Clobert, J. & Belichon, S. (1996). Phenotypic plasticity of growth and survival in the common lizard *Lacerta vivipara*. *Journal of Animal Ecology* **65**, 781-790.

Stamps, J. A. (1993). Sexual size dimorphism in species with asymptotic growth after maturity. *Biological Journal of Linnean Society* **50**, 123-145.

Stamps, J. A. & Andrews, R. M. (1992). Estimating asymptotic size using the largest individual per sample. *Oecologia* **92**, 503-512.

Stamps, J. A., Krishnan, V. V. & Andrews, R. M. (1994). Analyses of sexual size dimorphism using null growth-based models. *Copeia* **1994**, 598-613.

Stamps, J. A., Mangel, M. & Phillips, J. A. (1998). A new look at relationships between size at maturity and asymptotic size. *American Naturalist* **152**, 470-479.

Strijbosch, H. & Creemers, R. C. M. (1988). Comparative demography of sympatric populations of *Lacerta vivipara* and *L. agilis*. *Oecologia* **76**, 20-26.

Tertyshnikov, M. F. (2002). *Presmykayushchiesya Tsentral'nogo Predkavkaz'ya* /Reptiles of the Central Cis-Caucasia).' Stavropol: Stavropolservisshkola, 239 p. [in Russian].

Tinkle, D. W. & Dunham, A. E. (1986). Comparative life histories of two syntopic sceloporine lizards. *Copeia* **1986**, 1-18.

Wapstra, E., Swain, R. & O'Reilly, J. M. (2001). Geographic variation in age and size at maturity in a small Australian viviparous skink. *Copeia* **2001**, 646-655.

Warnecke, R. (2000). Auswertung erster Nachzuchtergebnisse von *Lacerta agilis boemica* Suchow, 1929 im Vergleich zu anderen Unterarten der Zauneidechse. *Die Eidechse* **11**, 28-38.

Watkins, G. C. (1996). Proximate causes of sexual size dimorphism in the iguanian lizard *Microlophus occipitalis*. *Ecology* **77**, 1473-1482.

Willemsen, R. E. & Hailey, A. (2001). Variation in adult survival rate of the tortoise *Testudo hermanni* in Greece: implications for evolution of body size. *Journal of Zoology, London* **255**, 43-53.

Wilson, D. S, Tracy, C. R. & Tracy, C. R. (2003). Estimating age of turtles from growth rings: A critical evaluation of the technique. *Herpetologica* **59**, 178-194.

Zakharov, V. M., Baranov, A. S. & Valetzky, A. V. (1982). Influence of incubation temperature on duration development of the sand lizard, *Lacerta agilis* (Lacertidae, Squamata). *Zoologichesky Zhurnal* **61**, 883-889.

Zamudio, K. R. (1998). The evolution of female-biased sexual size dimorphism: a population-level comparative study in horned lizards (*Phrynosoma*). *Evolution* **52**, 1821-1833.

Accepted: 1.6.05

APPENDIX 1. Geographical and climate characteristics of the study sites. Except Sergokala, there are no meteorological stations near the study sites, so only rough estimations of climate parameters drawn from coarse climatological maps (Atlas of the Daghestan ASSR, 1979) were possible. Inequalities show the position of a study site between two adjacent isolines of the corresponding climate parameter.

Locality	Geographical coordinates	Altitude (m)	Precipitation (P) April -October, mm	Annual evaporation (E), mm	Annual no. days with t>10°C (N)	Mean t of July (°C)
1. Kostek	43°20'N, 46°46'E	ca. 50	ca. 250	800<<E<1000	180<N<200	24
2. Sergokala	42°28'N, 47°42'E	550	ca. 300	800<E<1000	180<N<<200	22
3. Khuchni	41°57'N, 47°57'E	600	ca. 300	700<<E<800	ca.180	20<t<<24
4. Termenlik	42°25'N, 47°00'E	960	ca. 500	600<<E<700	140<<N<<180	16<<t<20
5. Kuli	42°01'N, 47°15'E	1900	500<P<600	600<E<700	140≤N<180	12<t<16

APPENDIX 2. Asymptotic SVL (A) and growth constant (k) of the von Bertalanffy growth model, and 80th percentiles of SVL (P80) of adult individuals for *Lacerta agilis boemica* and *L.strigata* from eight populations in the eastern North Caucasus. *The number of age-size values (data points in the scatter plots of Fig. 2); ** estimate ± asymptotic standard error; *** the number of adult (2-and-more years old) animals.

Study samples species	locality	sex	N*	A**	k**	R^2	n***	P80
agilis	Kostek	M	57	100.81±1.83	1.12±0.13	0.926	19	100.0
agilis	Kostek	F	55	89.12±5.00	0.88±0.27	0.839	17	88.0
agilis	Sergokala	M	32	91.51±2.36	0.97±0.12	0.815	17	90.0
agilis	Sergokala	F	25	77.11±2.55	2.76±1.22	0.963	14	83.8
agilis	Khuchni	M	42	98.50±3.29	1.00±0.13	0.947	19	94.0
agilis	Khuchni	F	30	92.24±2.71	1.46±0.23	0.966	13	91.0
agilis	Termenlik	M	48	95.22±1.08	0.97±0.06	0.967	14	97.0
agilis	Termenlik	F	70	89.04±1.17	1.10±0.10	0.925	15	94.2
agilis	Kuli	M	24	98.41±2.85	0.70±0.08	0.905	10	96.8
agilis	Kuli	F	33	95.36±1.12	0.96±0.06	0.957	11	97.0
strigata	Kostek	M	44	96.89±3.77	1.33±0.28	0.884	19	99.0
strigata	Kostek	F	43	93.01±3.03	1.26±0.24	0.889	18	92.4
strigata	Sergokala	M	29	98.82±2.64	0.86±0.09	0.956	14	96.0
strigata	Sergokala	F	22	91.61±2.01	1.31±0.12	0.986	13	84.0
strigata	Khuchni	M	33	94.55±3.90	1.02±0.16	0.940	19	89.0
strigata	Khuchni	F	41	91.39±1.95	1.12±0.12	0.951	15	92.0

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ECOMORPHOLOGICAL GUILDS IN ANURAN LARVAE: AN APPLICATION OF GEOMETRIC MORPHOMETRIC METHODS

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Ecomorphological guilds for anuran larvae are based on developmental modes, external morphology and habitat. Furthermore, several authors have investigated relationships between internal morphology and ecological habits. However, the relationships between internal morphology and tadpole ecological habits are not well established. In the present paper the quantitative methodology of geometric morphometrics is applied to look for correlation between the anatomy hyobranchial skeleton and the ecology of anuran larvae. Tadpoles of 14 species belonging to six different ecomorphological guilds were studied. The specimens were cleared and stained, and the hyobranchial apparatuses removed and drawn in ventral view. To record the shape variation, landmark-based geometric morphometric methodology was applied, involving a Relative Warp Analysis followed by multivariate statistics. Results show that species classify into four significantly different groups, according to their hyobranchial apparatus shape. Macrophagous tadpoles have well-developed ceratohyals and hypobranchial plate developed, and branchial baskets highly reduced. Generalized tadpoles have a large ceratobranchial area, with the hypobranchial plate covering a smaller area. Microphagous tadpoles have a very developed and complex branchial basket, and their hypobranchial plates are strongly reduced. Megalophagous tadpoles have the ceratohyals laterally expanded. These four groups are in general maintained after the inclusion of more species from the literature. Morphological groups can be related to size of food particles consumed, from very large in megalophages and macrophages, to very small, in highly efficient microphages.

Key words: feeding habit, frog, hyobranchial skeleton, morphology, tadpole

INTRODUCTION

Altig and Johnston (1989) define ecomorphological guilds for tadpoles on the basis of developmental modes, habitat, and external morphological characters, such as body shape, eye position, and oral disc configuration and orientation. They argue that it is possible to find general morphological patterns linked to specific habitats and feeding habits. Several researchers have found that not only external, but also internal morphology can be related to tadpole ecology. Thus, there exist a number of published studies on the relations between buccal, skeletal, and muscular characteristics and feeding habits (Wassersug, 1980; Satel & Wassersug, 1981; Hall *et al.*, 2002; Alcalde & Rosset, 2003; Vera Candioti & Haas, 2004; Vera Candioti *et al.*, 2004; Vera Candioti, 2005), and between internal morphology and microhabitat of these organisms (Noble, 1929; Haas & Richards; 1998).

Although most of the studies of tadpole chondrocrania are qualitative in nature, Larson (2002; 2005) analysed the ontogenetic changes in the chondrocranium of *Rana* spp. tadpoles, using a quantitative method for shape analysis. Geometric morphometric methods, followed by multivariate statistics, have been employed to show ontogenetic and allometric shape changes in other taxa (i.a., Monteiro & Abe, 1997; Monteiro *et al.*, 1999). In other studies, this methodology has been used to establish comparisons of shape among organisms of different species (i.e., Rohlf, 1993; Fink & Zelditch, 1995; Monteiro & Abe, 1999; Giri & Collins, 2004; Stayton, 2005). These methods quantify shape change and allow the visualisation of patterns of morphological change through the use of thin plate splines and vectors. This kind of analysis provides a valuable option by transposing the application of geometric morphometrics to shape variations among species with different morphologies linked to varied ecology. In this study, geometric morphometric methods are applied to the analysis of the shape variation in the hyobranchial skeleton of tadpoles. The hyobranchial apparatus constitutes the floor of the buccal cavity and supports the gill filters and gills. It relates directly to feeding, since it intervenes in the buccal pump mechanism and in sorting and entrapment of food particles.

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MATERIAL AND METHODS

Fourteen species of tadpoles from lentic environments were studied. They were selected according to the guilds mentioned by Altig & Johnston (1989). One to five individuals per species (46 specimens in total), at Gosner (1960) developmental stages 25-36 were analysed (see Appendix 1). The specimens were cleared and stained following the Wassersug (1976) protocol, then dissected using a stereomicroscope. The hyobranchial skeletons were removed and drawn in a ventral view, employing a camera lucida.

Variation in the shape of the hyobranchial skeletons across species was quantified following the geometric morphometric method described in Rohlf & Bookstein

TABLE I. Tadpole guilds according to Altig & Johnston (1989). *Telmatobius* cf. *atacamensis* is placed in this guild because, even though it breeds in mountain streams, its larvae live in small pools without currents.

CARNIVORES	
Ceratophrys cranwelli	Type I
Lepidobatrachus llanensis	Type II
MACROPHAGOUS	
Hyla nana	Type II
Hyla microcephala	
SUSPENSION FEEDERS	
Elachistocleis bicolor	Type II
Chiasmocleis panamensis	
SUSPENSION RASPERS	
Phyllomedusa hypochondrialis	
BENTHIC	
Bufo arenarum	
Bufo spinulosus	
Hyla rosenbergi	
Physalaemus santafecinus	
Telmatobius cf. *atacamensis*	
NEKTONIC	
Lysapsus limellus	
Scinax nasicus	

(1990) and Larson (2002). The removed hyobranchial skeletons were placed in a slide, trying to maintain the same orientation in each case. A set of landmarks was then marked on the right side of the skeleton, with the camera lucida. Landmark selection was based on Haas & Richards (1998), with some of the points redefined. The sixteen landmarks are (see also Fig. 1): (1) nostral margin of pars reuniens; (2) tip of processus anterior hyalis; (3) tip of processus anterolateralis; (4) articular condyle; (5) most lateral internal point of branchial basket; (6) most caudal point of gill slit I; (7) most caudal point of gill slit II; (8) most caudal and medial point of ceratobranchial III; (9) most nostral and medial point of ceratobranchial IV; (10) most caudal point of hypobranchial plates junction; (11) most caudal point of copula II; (12) lateral point of hypobranchial plate - copula II junction; (13) tip of processus posterior hyalis; (14) most caudal point of ceratobranchial I - hypobranchial plate junction; (15) most caudal point of ceratobranchial II - hypobranchial plate junction; (16) most caudal point of ceratobranchial III - hypobranchial plate junction.

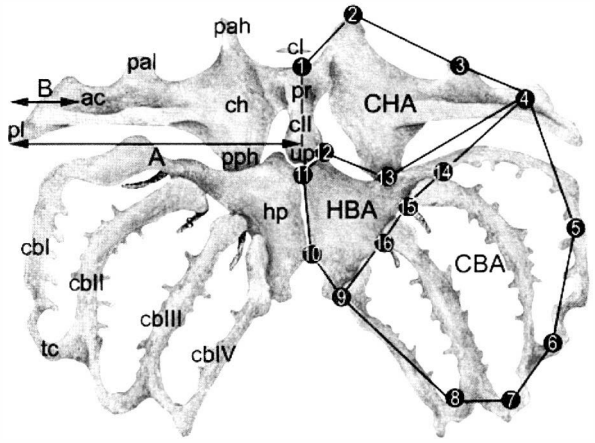

FIG. 1. Landmarks and measurements recorded on the hyobranchial skeleton, ventral view. 1-16, landmarks. Descriptions in text. A, width of ceratohyal; B, distance between the tip of the processus lateralis and the articular condyle; CBA, area of the ceratobranchials; CHA, area of the ceratohyal; HBA, area of the hypobranchial plate; ac, articular condyle; cb, ceratobranchial (I-IV); ch, ceratohyal; cl, copula I; cII, copula II; hp, hypobranchial plate; pah, processus anterior hyalis; pal, processus anterolateralis; pl, processus lateralis; pph, processus posterior hyalis; pr, pars reuniens; s, spiculum; tc, terminal commissure; up, urobranchial process.

The configurations of landmarks were next digitized, using the program tpsDig (Rolhf, 2004), and translated, standardised to centroid size=1, and aligned through the Generalized Procustes Analysis (GPA) to produce a consensus configuration, using the program tpsRelw (Rohlf, 2003). This method removes differences in size, position or rotation of the objects. A Relative Warp Analysis (i.e., Principal Component Analysis on the residuals from superimposition) was performed to obtain a plot of specimens scattered in a space defined by variability axes (the relative warps). Variation in shapes was depicted with thin-plate spline deformation grids, which reveals the modified shape compared to the consensus configuration (for further explanations see Rohlf & Bookstein, 1990; Bookstein, 1991; Fink & Zelditch, 1995; Monteiro & Reis, 1999; Adams *et al.*, 2004, among others). Finally, multivariate analyses were run (SPSS, 1998) on the scores of each specimen on the first relative warps, to test for significant differences among groups formed.

In addition to landmarks, some measurements already reported to be variable across species (Wassersug and Hoff, 1979; Haas & Richards, 1998) were recorded: in-lever arm proportion (distance between the lateral tip of processus lateralis and the articular condyle / total width of ceratohyal); ceratohyal area; hypobranchial area, ceratobranchials area (relative to the total hyobranchial apparatus area). Landmarks and measurements are shown in Fig. 1.

RESULTS

MORPHOLOGY OF THE HYOBRANCHIAL APPARATUS

Fig. 2 shows drawings of the hyobranchial apparatus of each of the 14 species. In *Bufo arenarum* tadpoles, the ceratohyals are elongated and have very prominent processes: processus anterior hyalis, anterolateralis, lateralis, posterior hyalis and the articular condyle, which is the point of articulation with the palatoquadrate. Copula I is small, and copula II is almost twice as long as the pars reuniens, and with a short urobranchial process. The ceratobranchials have numerous lateral projections and are distally joined by terminal commissures. Ceratobranchials I and II are synchondrotically attached to the hypobranchial plate,

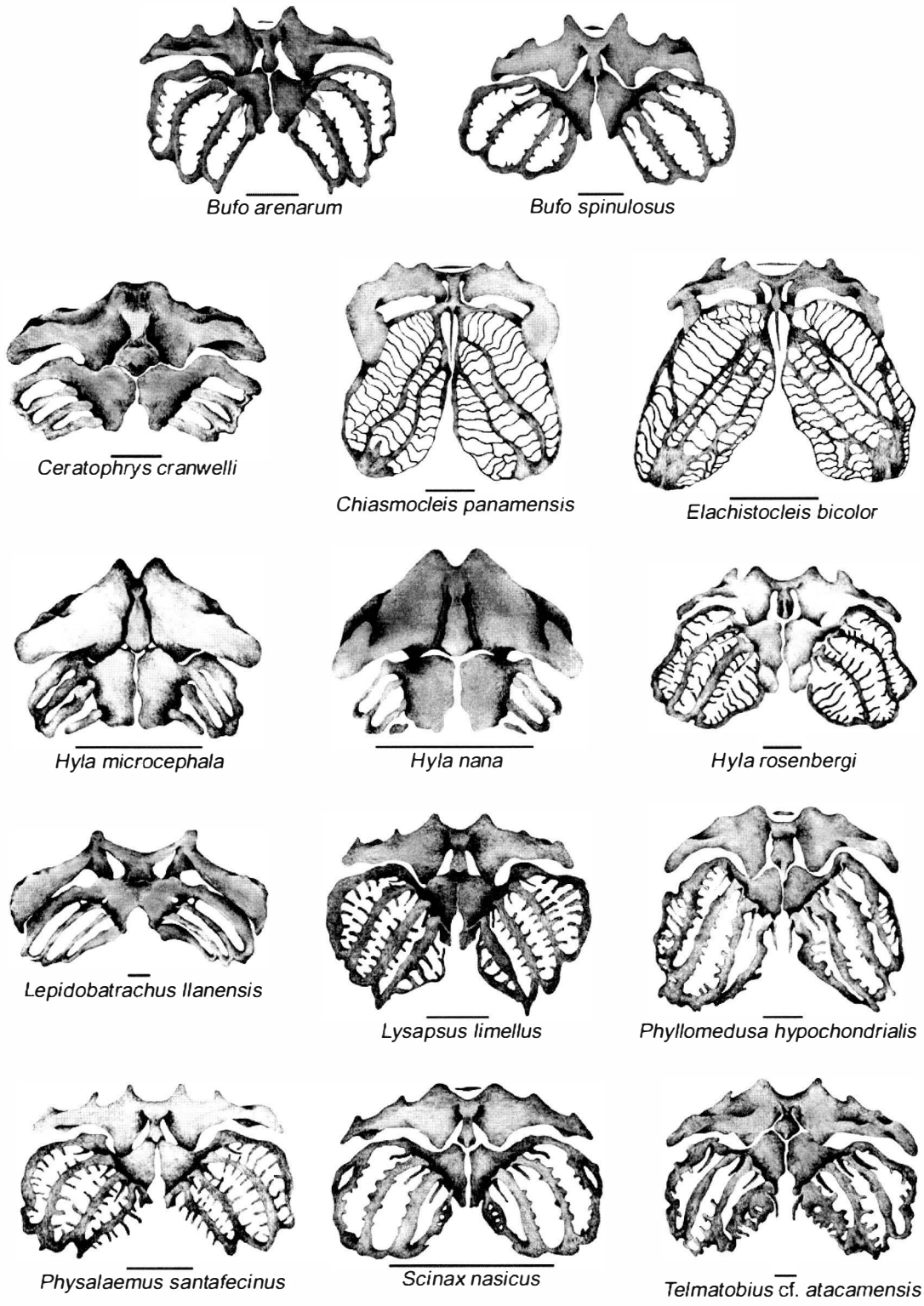

FIG. 2. Hyobranchial skeletons of the 14 species examined, ventral view. Scale bars =1mm.

and the III and IV are connected by conjunctive tissue. Dorsally, there are four spicula extending from the base of each ceratobranchial; the fourth one is reduced. Tadpoles of *B. spinulosus*, *Hyla rosenbergi*, *Physalaemus santafecinus*, *Scinax nasicus*, *Telmatobius* cf. *atacamensis*, *Lysapsus limellus* and *Phyllomedusa hypochondrialis* have very similar hyobranchial skeletons. In *L. limellus* the first three spicula are long and thin, and the fourth one forms a quadrangular, poorly chondrified plate, continuous with hypobranchial plate. *Phyllomedusa hypochondrialis* ceratohyals have small processus anterior hyalis and anterolateralis, and the urobranchial process is longer than in the other species (28% of the length of the copula II). Copula I is absent

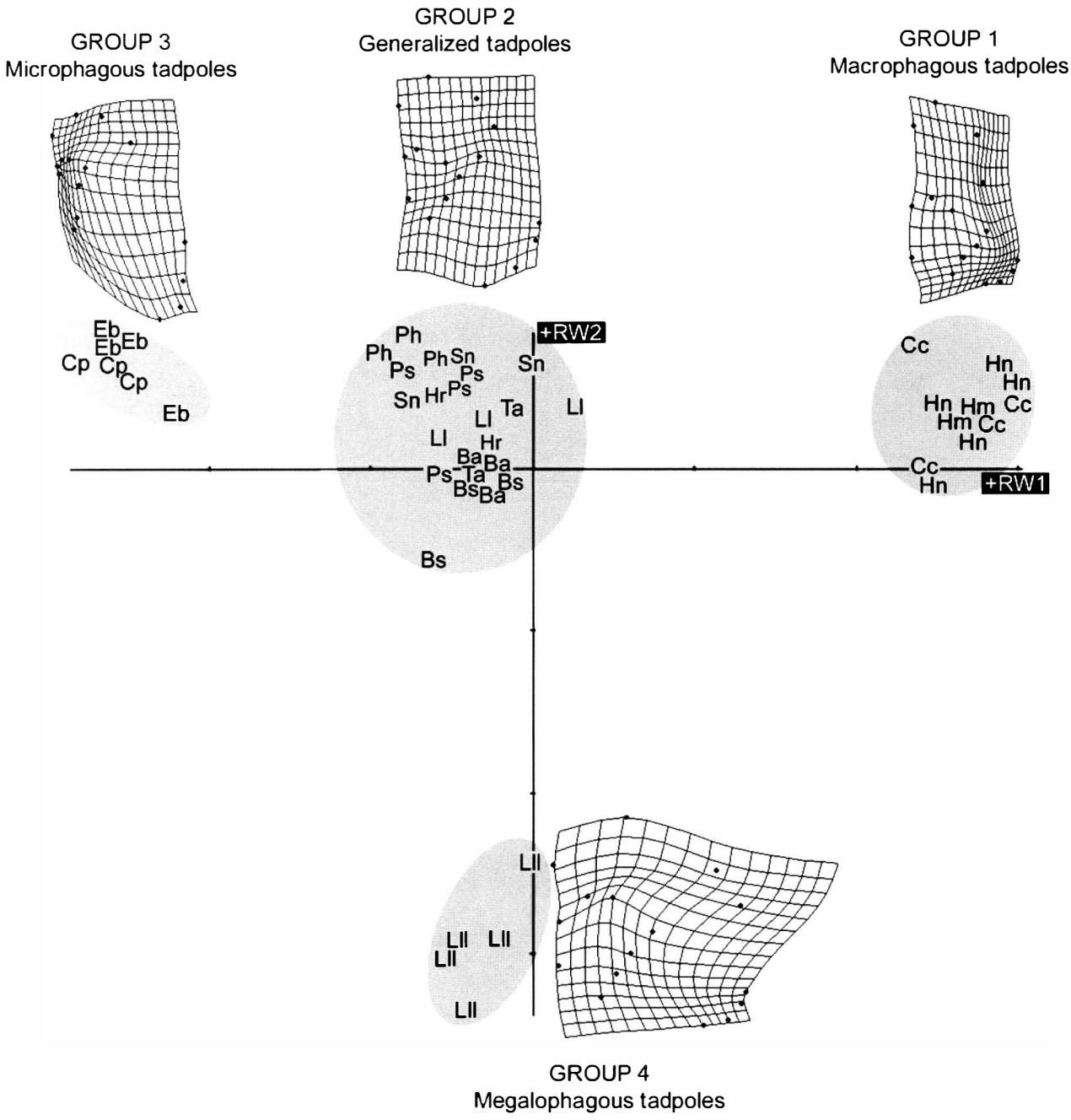

FIG. 3. Relative Warp Analysis of 14 species examined. Scores of 46 specimens on the first two relative warp axes. Shape variation among hyobranchial landmark configuration indicates four distinct clusters of specimens. Thin plate spline deformation grids describe shape variation at positive and negative extremes along each relative warp axis. The grids shown correspond to the highest and lowest scored specimen on each axis. Ba, *Bufo arenarum*; Bs, *Bufo spinulosus*; Cc, *Ceratophrys cranwelli;* Cp, *Chiasmocleis panamensis;* Eb, *Elachistocleis bicolor*; Hm, *Hyla microcephala*; Hn, *Hyla nana;* Hr, *Hyla rosenbergi;* Lll, *Lepidobatrachus llanensis;* Ll, *Lysapsus limellus*; Ph, *Phyllomedusa hypochondrialis;* Ps, *Physalaemus santafecinus*; Sn, *Scinax nasicus*; Ta, *Telmatobius cf. atacamensis*.

TABLE 2. Measurements of the hyobranchial skeleton of 14 species grouped in four clusters after the RW Analysis. Values are averages from the species within each group.

	In-lever arm proportion	Ceratohyal area	Hypobranchial area	Ceratobranchial area
Macrophagous tadpoles	0.51	44%	32%	24%
Generalized tadpoles	0.34	30%	20%	54%
Microphagous tadpoles	0.22	16%	7%	76%
Megalophagous tadpoles	0.21	35%	24%	41%

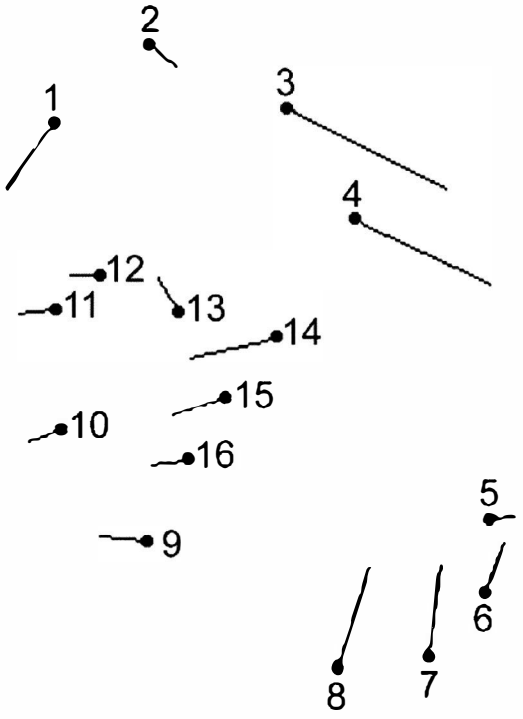

FIG. 4. Vector depiction of shape variation in *Lepidobatrachus llanensis* (megalophagous tadpoles) relative to the consensus. This species exhibit the lowest scores on the second relative warp. Black dots are landmarks of the consensus configuration and landmark numbers have been added to simplify interpretation. Note the longest vectors, showing the lateral translation of the ceratohyal processes, and the anterolateral translation of landmarks of the caudal region of the branchial basket.

in *H. rosenbergi*, *P. santafecinus*, *L. limellus* and *T.* cf. *atacamensis*.

In *Ceratophrys cranwelli*, the processes of the ceratohyals are short and massive, and the processus anterolateralis is not conspicuous. Copula I is absent and the ceratobranchials are devoid of lateral projections and spicula.

Hyla microcephala and *H. nana* have triangular and robust ceratohyals, with well-developed processus anterior hyalis, posterior hyalis, lateralis, and articular condyle. The processus anterolateralis is not conspicuous. Copula I and urobranchial process are absent. The pars reuniens is not clearly defined and appears vestigial, which makes the ceratohyals appear contiguous. The hypobranchial plates are thick, rectangular and continuous with copula II. The branchial basket is highly reduced; the ceratobranchials are bar-like, short, devoid of lateral projections and spicula. Terminal commissure III is absent in *H. microcephala* and some specimens of *H. nana*.

The microhylids *Chiasmocleis panamensis* and *Elachistocleis bicolor* have a very different hyobranchial apparatus. The processus lateralis is very developed and possesses a wide ventral laminar projection, caudally oriented. The processus posterior hyalis partially overlaps the proximal dorsal region of ceratobranchial I, and the articular condyle is a small protuberance on the lateral posterior margin of the ceratohyal. Copula I is a slender cartilaginous bar. The pars reuniens is continuous with the ceratohyals and copula II. Copula II is small and bears a very long, thin urobranchial process, approximately 1.5 times as long as the copula II. Caudally, copula II is fused to the hypobranchial plates, which are also fused together. The ceratobranchials are fused to the hypobranchial plates and constitute a large, complexly reticulated branchial basket. There are three long spicula, the first one wider, possibly resulting from fusion of spicula I and II.

Lepidobatrachus llanensis have highly elongated ceratohyals, laterally wider, and with prominent processus anterior and posterior hyales. The processus anterolateralis is located near the lateral edge. The pars reuniens is V-shaped, with thin branches fused to processes anteriores hyales. Copula I is absent, and copula II is wide, 1.6 times as long as the pars reuniens, and bears a small, quadrangular urobranchial process. The hypobranchial plates are medially fused, forming a structure with a concave caudal margin. The ceratobranchials are bar-like and joined by terminal commissures. There are neither lateral projections nor spicula.

GEOMETRIC MORPHOMETRIC ANALYSIS

Four clusters are detected by the Relative Warp Analysis, and are shown in the scatterplot of the first two relative warps (Fig. 3). The first relative warp (RW1) captures a high percentage of the total shape variation, 55.49%, accumulating 77.84% together with RW2. The species reported as macrophages and Type I carnivores score high on RW1, whereas the suspension-feeder microhylids are located at the opposite extreme, with the lowest values. Suspension-rasper, bentic and nektonic tadpoles score with intermediate values on RW1. All these three groups share high values on RW2. Finally, *Lepidobatrachus llanensis* constitutes a separate cluster, with intermediate and low values on RW1 and RW2 respectively. On the basis of hyobranchial apparatus shape, the species are clustered in four groups defined here as:

1. Macrophagous tadpoles: *Ceratophrys cranwelli, Hyla microcephala, Hyla nana;*
2. Generalized tadpoles: *Bufo arenarum, Bufo spinulosus, Hyla rosenbergi, Lysapsus limellus, Phyllomedusa hypochondrialis, Physalaemus santafecinus, Scinax nasicus, Telmatobius cf. atacamensis;*
3. Microphagous tadpoles: *Chiasmocleis panamensis, Elachistocleis bicolor;*
4. Megalophagous tadpoles (as mentioned by Ruibal & Thomas, 1988): *Lepidobatrachus llanensis.*

The deformation grids describe positive and negative deviations from the mean form along the relative warp axis. On the RW1 axis, they show that the main changes are associated with the position of landmarks 5-8 with respect to 9 and 14-16, i.e., the size of the branchial basket. From the consensus shape, which is very similar to

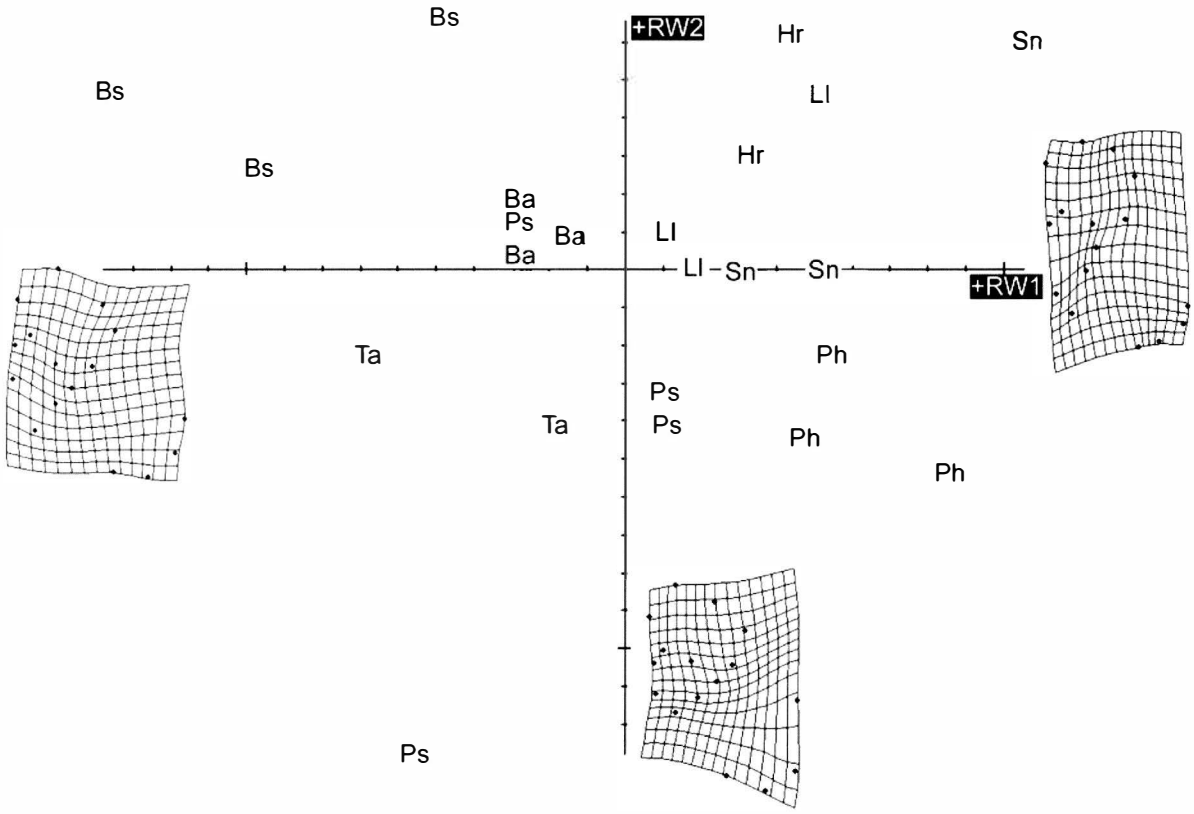

FIG. 5. Relative Warp Analysis of the generalist tadpoles group. Scores of 23 specimens on the first two relative warp axes. Ba, *Bufo arenarum*; Bs, *Bufo spinulosus*; Hr, *Hyla rosenbergi*; Ll, *Lysapsus limellus*; Ph, *Phyllomedusa hypochondrialis*; Ps, *Physalaemus santafecinus*; Sn, *Scinax nasicus*; Ta, *Telmatobius cf. atacamensis*. Variation of hyobranchial landmark configurations indicates no clusters. Thin plate spline deformation grids describe shape variation at positive and negative extremes along the relative warp axes. The grids correspond to the highest and lowest scored specimens.

the shape of generalized tadpoles, species with high scores on RW1 (macrophagous tadpoles: *Ceratophrys cranwelli* and *Hyla microcephala* group tadpoles) have reduced branchial baskets, whereas species with low scores (microphagous tadpoles: microhylids) have enlarged branchial baskets. Landmarks 9 and 14-16 also vary in their locations compared to landmarks 10-13, indicating a change in size of the hypobranchial plate from high to low scored species (macrophagous to microphagous tadpoles). On RW2, the main shape variation is due to landmarks 1-4, which indicate a lateral expansion of the ceratohyals in low scoring specimens. Landmark 12, which defines the width of the copula II, also varies, with its greatest width in *Lepidobatrachus llanensis*. Variation can also easily be observed using vector mode. For example, in the vector depiction of shape deviation from the consensus in *Lepidobatrachus llanensis*, the translation of landmarks 1-4, and 7-8 can be easily observed (Fig. 4).

A MANOVA was performed on the scores of each specimen on the first four relative warps, which accumulate almost 90% of the variation. The four groups differ significantly (Wilks' lambda = 0.0001; $P<0.001$). However, with *post hoc* tests, *Lepidobatrachus llanensis* (megalophagous tadpoles) is not different from generalized tadpoles on RW1 ($P=0.821$). On RW2, only *Lepidobatrachus llanensis* differs ($P<0.001$), whereas among the three remaining groups, there is significant difference between generalized and microphagous tadpoles ($P=0.021$), yet no difference between macrophagous and generalized tadpoles ($P=0.925$) nor between microphagous and macrophagous tadpoles ($P=0.131$).

A MANOVA was also performed on the measurements recorded from each specimen, using the four recently formed groups as a grouping variable. This analysis reveals significant differences among groups (Wilks'= 0.006; $P<0.001$). *Post hoc* tests show no significant differences between the in-lever arm proportion of *Lepidobatrachus llanensis* and microphagous tadpoles ($P=0.908$). A summary of the measurements in each group is shown in Table 2.

The generalized tadpoles group was considered in a separate analysis, and a RWA run (Fig. 5). This time, no definite groups are formed, and the first relative warps explain low and similar percentages of the total variation (RW1=30.44%; RW2=18.63%; RW3=12.48%; RW4=8.00%, totalling 69.55%). *Bufo* species and *Telmatobius* cf. *atacamensis* tend to score low on the RW1, whereas *Phyllomedusa hypochondrialis, Hyla rosenbergi and Scinax nasicus* show high values. *Physalaemus santafecinus* and *Lysapsus limellus* show intermediate values, overlapping both extreme groups. The first axis can be interpreted as a lateral and poste-

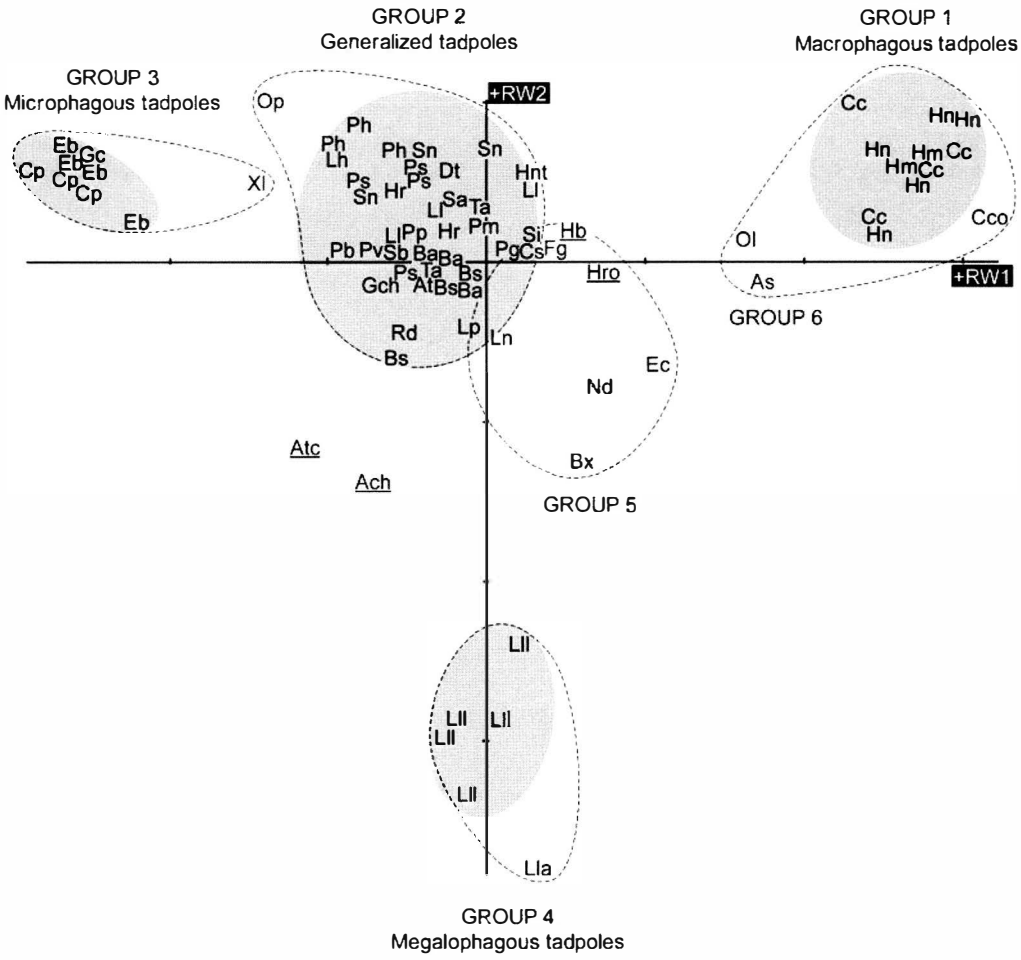

FIG. 6. Relative Warp Analysis of 14 species examined, plus 32 species from the literature (see Appendices; SET 1 and SET 2 respectively). Scores of the total of 78 specimens on the first two relative warp axes. SET 1: Ba, *Bufo arenarum*; Bs, *Bufo spinulosus*; Cc, *Ceratophrys cranwelli*; Cp, *Chiasmocleis panamensis*; Eb, *Elachistocleis bicolor*; Hm, *Hyla microcephala*; Hn, *Hyla nana*; Hr, *Hyla rosenbergi*; Lll, *Lepidobatrachus llanensis*; Ll, *Lysapsus limellus*; Ph, *Phyllomedusa hypochondrialis*; Ps, *Physalaemus santafecinus*; Sn, *Scinax nasicus*; Ta, *Telmatobius* cf. *atacamensis*. SET 2: As, *Anotheca spinosa*; Ach, *Atelophryniscus chrysophorus*; At, *Ascaphus truei*; Atc, *Atelopus tricolor*; Bx, *Boophis* sp.; Cco, *Ceratophrys cornuta*; Cs, *Cycloramphus stejnegeri*; Dt, *Dendrobates tinctorius*; Ec, *Eupsophus calcaratus*; Fg, *Flectonotus goeldii*; Gc, *Gastrophryne carolinensis*; Gch, *Gastrotheca christiani*; Hb, *Hymenochirus boettgeri*; Hnt, *Heleophryne natalensis*; Hro, *Hoplophryne rogersi*; Lh, *Leptobrachium hasselti*; Lla, *Lepidobatrachus laevis*; Ln, *Litoria nannotis*; Lp, *Leptodactylus pentadactylus*; Nd, *Nyctimystes dayi*; Ol, *Occidozyga laevis*; Op, *Otophryne pyburni*; Pb, *Phyllomedusa boliviana*; Pg, *Phasmahyla guttata*; Pm, *Pseudis minuta*; Pp, *Pseudis paradoxa*; Pv, *Phrynohyas venulosa*; Rd, *Rhinoderma darwinii*; Sa, *Scinax acuminatus*; Sb. *Scaphiopus intermontanus*; Sp, *Spea bombifrons*; Xl, *Xenopus laevis*. Grey ellipses are the original four groups from Fig. 3. Dash-lines are groups formed on the basis of probabilities of group membership (see Discussion for further explanations). Underlined names are the four species with a 10-landmark configuration. Note the location of most of the species within the original groups.

rior translation of processes anteriores hyales and anterolaterales, and the articular condyles, plus a slight widening of the branchial basket, due to the lateral translation of landmarks 5-8, from high to low scored specimens. The RW2 axis indicates an enlargement of the branchial basket, as a consequence of the posterior translation of the landmarks 6-8, in low scored specimens.

In order to test if the groups formed were also maintained after the inclusion of more specimens, a second set of species was added, consisting of published pictures of the hyobranchial apparatus of several taxa (a list of the species and their references is given in Appendix 2). A variety of species were included, in an attempt to complete the remaining guilds. Nonfeeding tadpoles were also considered. All these illustrations were treated as mentioned above. The results are shown in Fig. 6, which shows a scatterplot of the specimens on the first two relative warps. Most of the added species fall into the four groups defined in the former analysis. *Lepidobatrachus laevis* groups with *L. llanensis* within megalophagous tadpoles; *Gastrophryne carolinensis* groups within the previously examined microphagous tadpoles; *Ceratophrys cornuta* fits with the macrophagous tadpoles, and most of the remaining species distribute among generalized tadpoles. Other species are not placed in the established groups. To determine if they constitute separate clusters in their own right, a Discriminant Analysis was performed on the scores of the species on the first four relative warps.

Group membership was determined a priori on the basis of the four groups formed by the RWA. Doubtful species were coded as separate groups, and probabilities of group membership and predicted group were saved. The species to confirm were: *Boophis* sp., *Eupsophus calcaratus* and *Nyctimystes dayi,* considered as Group 5, and *Anotheca spinosa* and *Occidozyga laevis*, as Group 6. The analysis confirms the new groups, with high probability values (P>0.97 in all five cases), and lower probabilities that the species belonged to Groups 2 and 1 respectively. Additionally, three species were misclassified. They are *Xenopus laevis*, coded as Group 2 (generalized tadpoles), but assigned to Group 3 by the analysis (microphagous tadpoles; P=0.99), and *Flectonotus goeldii* and *Litoria nannotis*, coded as Group 2, but assigned to the new Group 5, although this time with lower P-values (P=0.53 and P=0.54 respectively).

A constraint on landmark-based geometric morphometric methodology is that it requires a complete and comparable set of landmarks, thus precluding the study of the origination or elimination of structures. Focusing only on landmarks common to all specimens can clearly affect the results of such a study (Adams *et al.*, 2004). Bookstein & Smith (2000) offer a possible solution to the appearance of new structures, but this method is not yet generalised. Consequently, in the present paper, tadpoles of *Atelopus tricolor*, *Atelophryniscus chrysophorus* (gastromizophorous), *Hymenochirus boettgeri* (Type III carnivore), and *Hoplophryne rogersi* (Arboreal Type I), even when there are available pictures in the literature, could not be included at the start, because all of them lack 1-3 ceratobranchials and thus landmarks 6-9 and 15-16. However, because it is of interest to analyse where these species would locate with relation to the remaining taxa, a second analysis was performed to include them, eliminating the said landmarks. Although such analysis entails loss of information due the elimination of landmarks, it results in a distribution of the species almost identical to the original one, implying that morphological shape variation can be captured by examining only the ceratohyals, hypobranchial plates and the first ceratobranchial. The composition of the microphagous, generalized, and megalophagous tadpoles groups remains unmodified, the only difference in macrophagous tadpoles group being the location of *Occidozyga laevis* (closer to macrophagous tadpoles), and of *Anotheca spinosa*, which moves away from this group to align itself with the most marginal sector of the generalized tadpoles group. In order to avoid repetition of figures, the four mentioned species have been added to Fig. 6 (names underlined), to show the approximate position where their 10-landmark configurations place them. *Hymenochirus boettgeri* and *Hoplophryne rogersi* locate in the 'macrophagous' extreme of the generalized tadpoles group, whereas gastromizophorous larvae fall between generalized and megalophagous tadpoles.

DISCUSSION

Some of the groups formed by RWA in the first analysis are in fact quite expected. A glance at the hyobranchial apparatus of macrophagous tadpoles (*Ceratophrys cranwelli* and *Hyla microcephala* group tadpoles) versus microhylids for example, suggests that these species would be placed in different groups. Generalized tadpoles include all the other species that are neither macrophagous nor microhylids tadpoles. The exception is *Lepidobatrachus llanensis*, which constitutes its own group, rather than clustering with the macrophagous tadpoles. The thin plate splines reveal that the ceratohyals of this species is different enough for it to justify a separate group. *Lepidobatrachus llanensis* tadpoles in fact, possess a mixture of both microphagous microhylids (low value of in-lever arm proportion) and generalized tadpoles (relative areas of the hyobranchial apparatus) characteristics.

After the inclusion of the second set of species taken from the literature, the original four groups are generally maintained. Group 6 (*Occidozyga laevis* and *Anotheca spinosa*) could probably be linked to the macrophagous tadpoles group, with the addition of more species and the subsequent translation of the centroids of both groups. In Group 5 this tendency is more pronounced, as visualised in the analysis of species located near the margins of the cluster: *Flectonotus goeldii* and *Litoria nannotis* show similar probabilities values 50%) of being assigned to Groups 5 and 2 (i.e., to be considered as a new group or clustered with the generalized tadpoles). This results in Group 5 appearing to include itself within Group 2. It is thus possible to visualise a sort of continuum within this large Group 2, with suctorial and nidicolous tadpoles (viz., *Boophis sp.*, *Eupsophus calcaratus, Nyctimystes dayi*) located on one of the extremes, toward microphagous and megalophagous tadpoles groups, and psammonic, fossorial and suspension-raspers (viz., *Otophryne pyburni*, *Leptobrachium hasselti, Phyllomedusa hypochondrialis*) tending to the opposite extreme, near microphagous tadpoles. Conversely, Groups 2 and 3 (generalized and microphagous tadpoles groups) remain well defined, because the most marginal species (*Xenopus laevis* and *Otophryne pyburn*i) show high probability of membership in their own groups (P=0.99 and P=0.97 respectively).

Considering all of the above, how are these results to be interpreted in the context of the Altig and Johnston's ecomorphological guilds for tadpoles? To begin with, the 18 groups defined on the basis of external morphology and habitat could be consolidated into four to six (depending on the strict consideration of Groups 5 and 6) groups based on hyobranchial skeleton morphology. This conclusion is preliminary though, because I did not include enough samples from all 18 Altig and Johnston's groups. The tentative four groups are as follows:

1. *Macrophagous tadpoles.* It includes Altig and Johnston's Type II macrophages *(Hyla nana* and *H. microcephala)* some Type I carnivores (*Ceratophrys cranwelli* and *C. cornuta*) and probably, Type I macrophages and Type II arboreal (Group 6; *Occidozyga laevis* and *Anotheca spinosa*).
2. *Generalized tadpoles.* Considering Group 5, it includes all the endotrophs (exoviviparous, paraviviparous, and nidicolous: *Rhinoderma darwinii*, *Gastrotheca christiani*, *Eupsophus calcaratus*, *Cycloramphus stejnegeri* and F*lectonotus goeldii*), and among exotrophs, clasping (*Boophis* sp.), suctorial (*Ascaphus truei, Heleophryne natalensis*, *Nyctimystes dayi* and *Litoria nannotis*), neustonic (*Phasmahyla guttata*), psammonic (*Otophryne pyburni)*, Type II fossorial (*Leptobrachium hasselti*), Type I and unidentified Type arboreal (*Hoplophryne rogersi* and *Dendrobates tinctorium*), some Type I and Type III carnivores (*Leptodactylus pentadactylus* and *Spea bombifrons,* and *Hymenochirus boettgeri*), nektonic *(Lysapsus limellus, Phrynohyas venulosa, Pseudis minuta, P. paradoxa, Scinax acuminatus* and *S. nasicus*), benthic (*Bufo arenarum, B. spinulosus, Hyla rosenbergi, Physalaemus santafecinus, Scaphiopus intermontanus* and *Telmatobius* cf. *atacamensis*), and suspension-rasper (*Phyllomedusa boliviana* and *P. hypochondrialis*). Gastromizophorous tadpoles (*Atelopus tricolor* and *Atelophryniscus chrysophorus*) seem to cluster with this group also.
3. *Microphagous tadpoles.* It includes suspension-feeders (*Chiasmocleis panamensis*, *Elachistocleis bicolor, Gastrophryne caroliniensis* and *Xenopus laevis*).
4. *Megalophagous tadpoles.* It includes Type II macrophages (*Lepidobatrachus laevis* and *L. llanensis).*

Wassersug & Hoff (1979) also detected four groups by analysing several measurements of the hyobranchial apparatus, in-lever arm proportion, buccal cavity volume, and angle of rotation of the ceratohyal, but *Lepidobatrachus* spp. were not included, and the fourth group is composed of benthic and torrent tadpoles. With the methodology applied in the present paper, and considering shape variables, such larvae do not differ significantly from generalized tadpoles.

The species in the macrophagous tadpoles group are characterised mainly by the reduced size of the branchial basket and large ceratohyals and hypobranchial plates. Additionally, in-lever arm proportion values are the highest of all groups. For tadpoles to ingest large food items requires either a large buccal aperture and buccal cavity, or a jaw apparatus capable of tearing the prey apart before engulfing it. The species in the macrophagous tadpoles group share the fact that they have achieved a large buccal cavity volume by the enlargement of the buccal floor. Tadpoles of the *Hyla microcephala* group, *Ceratophrys* spp., *Anotheca spinosa* and *Occidozyga laevis* possess very robust ceratohyals, with greater nostrocaudal length than that of the hypobranchial plate. These features are in most of the species accompanied by a conspicuous development of the musculature responsible for depressing the buccal floor (Satel & Wassersug, 1981; Vera Candioti *et al.*, 2004; Vera Candioti, 2005).

With regard to feeding habits, all of these species consume large items, compared to the microscopic particles typically found in the guts of generalized tadpoles. *Ceratophrys cranwelli,* for instance, feeds on varied items whose maximum diameters represent 50% of the snout-vent length of the tadpole (Vera Candioti, 2005). *Hyla nana* tadpoles have a diet mainly composed of oligochaetes with average length that may represent 120% of the tadpole length (Vera Candioti *et al.*, 2004; pers. obs.). *Hyla microcephala* tadpoles examined in this study also had oligochaetes in their gut contents, and Wassersug and Hoff (1979) reported large filamentous plants in their diet. Finally, *Anotheca spinosa* and *Occidozyga laevis* are also macrophagous, sometimes oophagous (Jungfer, 1996; Altig & McDiarmid, 1999).

In the megalophagous *Lepidobatrachus*, the hyobranchial skeleton has large lateral extension. The branchial basket, even if it has bar-like ceratobranchials with neither spicules nor lateral projections, is not reduced in area. In this genus, unlike macrophagous tadpoles, the large buccal cavity volume is achieved by widening the whole body. The large lateral extension of the ceratohyals is, in fact, part of a lateral extension of the anterior and middle regions of the chondrocranium. The wide suprarostral and the lower jaw, both laterally expanded, create a large mouth opening. The lateral extension of palatoquadrates and ceratohyals, in turn, yield a large buccal volume. This implies that prey can be not only exceptionally large (nearly equal to the predator size), but can also be engulfed intact. Despite the low in-lever arm proportion value, the m. orbitohyoideus (responsible for the descent of the buccal floor) is very developed, inserting on the anterior margin of the quadratocranial commissure (Palavecino, 1999; pers. obs.). This indicates the generation of a strong negative pressure inside the buccal cavity. Ruibal & Thomas (1988) report for *Lepidobatrachus* spp. a diet mainly composed of live prey such as *Artemia*, worms, tadpoles and small fishes.

Most of the species fall into the generalized tadpoles group. Around 50% of the total area of the hyobranchial apparatuses is occupied by the branchial basket, and the hypobranchial area is smaller than the ceratohyal area. Mean in-lever arm proportion is 0.34, consistent with what is reported in Wassersug & Hoff (1979). As already mentioned, it is possible to detect a general tendency within this group, explained mainly by habitat. The species inhabiting fast water locate at one extreme, near macrophagous tadpoles. A similarity between hyobranchial skeletons of suctorial and macrophagous

larvae has been reported, and is explained by the fact that both kinds of tadpoles generate high negative pressures inside their buccal cavities, either to capture and retain large prey, or to adhere to the substrate (see Wassersug & Hoff, 1979; Haas & Richards, 1998). At the opposite extreme, near the microphagous tadpoles, are species with low mobility, inhabitants of lentic environments, or slow water microhabitats in lotic environments. Morphologically, the spectrum just described is characterised by a progressive increase of the size of the branchial basket, which results in an increase of the filtering capability for feeding. The 'macrophagous' extreme joins some of the species without active feeding, and suctorial tadpoles. Haas & Richards (1998) mentioned whole ephemeropteran larvae inside the digestive tract of *Boophis* sp. *Ascaphus truei* tadpoles, which are also suctorial but with a less modified hyobranchial apparatus, scrape algae from the rocks they cling to (Wassersug, 1972). Some endotrophic larvae have reduced branchial baskets (Lavilla, 1991; Haas, 1996*a*; Vera Candioti *et al.*, 2005); like macrophagous tadpoles, they do not depend on an efficient filtering mechanism to obtain their food. At the 'microphagous' extreme are suspension-rasper tadpoles, which generate a suspension of small particles (*Phyllomedusa hypochondrialis*, mean=1.7% of the snout-vent length; pers. obs.), and *Otophryne pyburni*, known to filter microscopic particles from the sandy bottom where it lives (Wassersug & Pyburn, 1987). The remaining species of the generalized tadpoles group are mostly benthic, nektonic, and neustonic, feeding on small particles obtained in various ways and from different microhabitats (i.e., *Bufo spinulosus* mean = 1.8% of the snout-vent length; *Physalaemus santafecinus* mean = 5.5% of the SVL; *Telmatobius* cf. *atacamensis* mean =13% of the SVL; pers. obs.). Also included here is the enormous tadpole of *Pseudis paradoxa* which, despite its size, has a generalized diet, mainly composed of macrophyte fragments, arthropod remnants and microalgae, with sizes that represent 11% of the snout-vent length (Rada & Bello, 1988; Arias *et al.*, 2002; pers. obs.).

Some species show that the concordance among feeding habit and morphology does not always hold. *Rhinoderma darwinii* and *Gastrotheca christiani* are endotrophic species, which retain a hyobranchial apparatus similar to that of the generalized tadpoles (Lavilla, 1987; Lavilla & Vaira, 1997), instead of the reduced one of other endotrophic tadpoles (i.e., *Cycloramphus stejnegeri, Flectonotus goeldii, Eupsophus calcaratus*; Lavilla, 1991; Haas, 1996*a*; Vera Candioti *et al.*, 2005). *Leptodactylus pentadactylus* are facultatively carnivorous tadpoles (Heyer *et al.*, 1975), yet maintain a generalized hyobranchial apparatus, very similar to those of most of the species of the genus (Larson & De Sá, 1998). Finally, *Spea bombifrons* can be a macrophagous carnivore too, and although its buccal volume, ceratohyal development and in-lever arm proportion are all similar to those of macrophagous tadpoles (Wassersug & Hoff, 1979), its branchial basket is not reduced enough to cluster it with them. The genera *Spea* and *Scaphiopus* are an interesting case, because some species have both generalized and carnivorous morphs, the latter characterised by modifications in the oral apparatus, intestinal length, fat bodies, and buccal floor levator and depressor muscles (Bragg, 1956; Bragg & Bragg, 1959; Acker & Larsen, 1979; Wassersug & Hoff, 1979; Hall & Larsen, 1998; Hall *et al.*, 2002). The location within generalized tadpoles group of both *Spea bombifrons* (carnivorous morph) and *Scaphiophus intermontanus* (herbivorous morph) suggests that apparently macrophagy does not necessitate major changes in the hyobranchial skeleton of these taxa.

The four species not included in the complete 16-landmark analysis apparently fit into the generalized tadpoles group. The location of gastromizophorous tadpoles, near *Lepidobatrachus*, may be explained by the lateral expansion of the hyobranchial skeleton, also accompanied in this case by a lateral expansion of the whole chondrocranium (Lavilla & De Sá, 2001). However, since the mouth opening is much smaller in gastromizophorous larvae than in *Lepidobatrachus* sp. (as inferred from mandible width), the lateral expansion of the head seems to be related more to the presence of a belly sucker presence than to the size of the food items ingested by these tadpoles. Data on the diet of these species would be needed in order to confirm this hypothesis. *Hoplophryne rogersi* tadpoles are macrophagous; their gut contents are mainly debris from arthropods and vegetal tissues, plus frog eggs (Noble, 1929). *Hymenochirus boettgeri* tadpoles are macrophagous too; they pursue and ingest live prey such as microcrustaceans, *Culex* larvae, and also small tadpoles (Sokol, 1962; Deban & Olson, 2002). These two latter species are located at the 'macrophagous' extreme of the generalized tadpoles group.

Finally, microphagous group tadpoles possess a highly developed branchial basket, with a relative area greater than 70% of the total hyobranchial area. The hypobranchial area is greatly reduced. The low in-lever arm proportion for tadpoles of this group is consistent with what Wassersug & Hoff (1979) reported. These authors calculated a large angle of rotation of the ceratohyal during the descent of the buccal floor. This allows microphagous tadpoles to draw large volumes of water into the buccal cavity without having to generate high negative pressure. Additionally, the region of mucous secretory ridges is extensive in these tadpoles, implying a more efficient mucous entrapment process (Seale & Wassersug, 1979).

Elachistocleis bicolor consume especially small particles (0.03 mm - 0.06 mm; <1% - 1% of the SVL; pers. obs.). Wassersug (1972) explored the efficiency of some species of tadpoles removing solid particles of different sizes from a suspension. *Xenopus laevis* tadpoles are capable of filtering the smallest particles (0.126 μ), in comparison with generalized tadpoles

(*Ascaphus truei* and *Rana pipiens*, 0.557 μ). Due to the similarity between most microhylids and *Xenopus laevis* branchial baskets, it seems probable that *Elachistocleis*, *Chiasmocleis* and *Gastrophryne* can also feed on particles smaller than 0.03 mm. This kind of highly efficient microphagy seems to be exclusive to microhylids, pipids and rhinophrynids. Although microhylid species included in this work are few, the morphological constancy in the hyobranchial skeleton across the family would seem to indicate that new taxa included would fit within this group. Exceptions with a less specialised hyobranchial skeleton (besides *Hoplophryne rogersi*) could be *Microhyla heymonsi*, a neustonic form capable of macrophagy, and *Scaphiophryne* spp., reported to be bottom grazers and carnivorous (Blommer-Schlösser, 1975; Wassersug, 1984; 1989; Altig & McDiarmid, 1999). *Rhinophrynus dorsalis*, with a hyobranchial skeleton similar to that of microhylids (Swart & De Sá, 1999), is both a proficient suspension-feeder and a predator of large zooplankton (Starrett, 1960).

In sum, macrophagy seems possible across a very broad array of hyobranchial skeletal morphologies. First, it occurs in macrophagous and 'near' generalized tadpoles, associated with very developed, robust, and long ceratohyals, and a marked reduction of the branchial basket. Secondly, it occurs in megalophagous species (*Lepidobatrachus* spp.), where the large buccal cavity volume is a result of the widening of the hyobranchial skeleton and neurocranium with the hyobranchial basket only being slightly reduced. Thirdly, it occurs among generalized tadpoles, with a scarcely or unmodified hyobranchial skeleton. In this latter case, the large buccal cavity volume results from a simple enlargement of the body size, with an absolute value that increases exponentially with linear measurements such as snout-vent length and width at eyes (Wassersug & Hoff, 1979). This also coincides with the observations of Petranka & Kennedy (1999), who discuss the capability of a number of generalized-morphology tadpoles to ingest large prey. Finally, some forms with a hyobranchial apparatus typically characterised as microphagous, such as *Rhinophrynus dorsalis* and *Microhyla heymonsi*, are also capable of macrophagy. There is a strong relation between macrophagy and muscular features. Most of the species feeding on large prey have an extensive development of the buccal floor depressor muscles, regardless of the configuration of their hyobranchial skeleton (Satel & Wassersug, 1981). An example is *Spea bombifrons*, with a very low IH/OH ratio (mm. interhyoideus / mm. orbitohyoideus; buccal floor levator and depressor muscles respectively) in the carnivorous morph, but similar to the generalized tadpoles in the omnivorous morph. On the contrary, highly efficient microphagy would only occur in tadpoles with large branchial baskets, which support complex filtering and particle entrapment structures.

The groups formed on the basis of hyobranchial morphology can be generally related to size of food particles. Species for which data on diet are available locate in different groups, showing a tendency for a decrease in average food-particle size, from very large within macrophagous tadpoles, to very small in microphagous forms. Additional work must be done to further address the question of whether these morphological groups effectively correlate with a gradient of food-particle sizes.

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REFERENCES

Acker, R. L. & Larsen, J. H. Jr. (1979). A functional analysis of morphological variation in larval *Scaphiopus intermontanus*. *American Zoologist* **19**, 1012.

Adams, D. C., Rohlf, F. J., & Slice, D. E. (2004). Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology* **71**, 5-16.

Alcalde, L. & Rosset, S. D. (2003). Descripción y comparación del condrocráneo en larvas de *Hyla raniceps* (Cope, 1862), *Scinax granulatus* (Peters, 1871) y *Scinax squalirostris* (A. Lutz, 1925) (Anura: Hylidae). *Cuadernos de Herpetología* **17**, 33-49.

Altig, R. & Johnston, G. F. (1989). Guilds of anuran larvae: relationships among developmental modes, morphologies and habitats. *Herpetological Monographs* **3**, 81-109.

Altig, R. & McDiarmid, R. W. (1999). Diversity. Familial and generic characterisation in tadpoles. In *The biology of anuran larvae*, 295-337. McDiarmid, R. W. & Altig, R. (Eds). Chicago: The University of Chicago Press.

Arias, M. M., Peltzer, P. M., & Lajmanovich, R. C. (2002). Diet of the giant tadpole *Pseudis paradoxa platensis* (Anura: Pseudidae) from Argentina. *Phyllomedusa* **1**, 97-100.

Blommers-Schlösser, R. M. A. (1975). Observations on the larval development of some Malagasy frogs, with notes on their ecology and biology (Anura; Dyscophynae, Scaphiophryninae and Cophylinae). *Beaufortia* **24**, 7-26.

Bookstein, F. L. (1991). Morphometric tools for landmarks data. Cambridge: Cambridge University Press.

Bookstein, F. L. & Smith, B. R. (2000). Inverting development: geometric singularity theory in embryology. In *Mathematical modelling, estimation and imaging*, 139-174. Wilson, D., Tagare, H., Bookstein, F. L., Préteauz, F. & Dougherty, E. (Eds). Washington: Proc. SPIE. vol. 4121.

Bragg, A. N. (1956). Dimorphism and cannibalism in tadpoles of *Scaphiopus bombifrons* (Amphibia: Salientia). *Southwestern Naturalist* **1**, 105-108.

Bragg, A. N. & Bragg, W. N. (1959). Variation in the mouth parts in tadpoles of *Scaphiopus* (Spea) *bombifrons* Cope (Amphibia: Salientia). *Southwestern Naturalist* **3**, 55-59.

Deban, S. M. & Olson, W. M. (2002). Suction feeding by a tiny predatory tadpole. *Nature* **420**, 41-42.

Fabrezi, M. & Lavilla, E. O. (1992). Estructura del condrocráneo y esqueleto hiobranquial en larvas de algunos hílidos neotropicales (Anura: Hylidae). *Acta Zoológica Lillonana* **41**, 155-164.

Fabrezi, M. & Vera, R. (1997). Caracterización morfológica de larvas de anuros del Noroeste argentino. *Cuadernos de Herpetología* **11**, 37-49.

Fink, W. L. & Zelditch, M. L. (1995). Phylogenetic analysis of ontogenetic shape transformations: a reassessment of the piranha genus *Pygocentrus* (Teleostei). *Systematic Biology* **44**, 343-360.

Giri, F. & Collins, P. A. (2004). A geometric morphometric analysis of two sympatric species of the family Aeglidae (Crustacea, Decapoda, Anomura) from La Plata basin. *Italian Journal of Zoology* **71**, 85-88.

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

Haas, A. (1995). Cranial features of dendrobatid larvae (Amphibia: Anura: Dendrobatidae). *Journal of Morphology* **224,** 241-264.

Haas, A. (1996*a*). Non-feeding and feeding tadpoles in hemiphractine frogs: larval head morphology, heterochrony, and systematics of *Flectonotus goeldii* (Amphibia: Anura: Hylidae). *Journal of Zoological Systematics and Evolutionary Research* **34**, 163-171.

Haas, A. (1996*b*). The larval hyobranchial apparatus of discoglossoid frogs: its structure and bearing on the systematics of the Anura (Amphibia: Anura) *Journal of Zoological Systematics and Evolutionary Research* **35**, 179-197.

Haas, A. (2003). Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics* **19**, 23-89.

Haas, A. & Richards, S. J. (1998). Correlations of cranial morphology, ecology, and evolution in Australian suctorial tadpoles of the genera *Litoria* and *Nyctimystes* (Amphibia: Hylidae: Pelodryadinae). *Journal of Morphology* **238,** 109-141.

Hall, J. A. & Larsen, J. H. Jr. (1998). Postembryonic ontogeny of the Spadefoot toad, *Scaphiopus intermontanus* (Anura: Pelobatidae): skeletal morphology. *Journal of Morphology* **238**, 179-244.

Hall, J. A., Larsen, J. H. Jr. & Fitzner, R. E. (2002). Morphology of the prometamorphic larva of the Spadefoot toad, *Scaphiopus intermontanus* (Anura: Pelobatidae), with an emphasis on the lateral line system and mouthparts. *Journal of Morphology* **252**, 114-130.

Heyer, W. R., McDiarmid, W. R. & Weigmann, D. L. (1975). Tadpoles, predation and pond habitats in the tropics. *Biotropica* **7**, 100-111.

Jungfer, K. H. (1996). Reproduction and parental care of the Coronated treefrog *Anotheca coronata* (Steindachner 1864) (Anura: Hylidae). *Herpetologica* **52**, 25-32.

Larson, P. M. (2002). Chondrocranial development in larval *Rana sylvatica* (Anuran: Ranidae): morphometric analysis of cranial allometry and ontogenetic shape change. *Journal of Morphology* **252**, 131-144.

Larson, P. M. (2005). Ontogeny, phylogeny, and morphology in anuran larvae: morphometric analysis of cranial development and evolution in *Rana* tadpoles (Anura: Ranidae). *Journal of Morphology* **264**, 34-52.

Larson, P. M. & De Sá, R. O. (1998). Chondrocranial morphology of *Leptodactylus* larvae (Leptodactylidae: Leptodactylinae): its utility in phylogenetic reconstruction. *Journal of Morphology* **238**, 287-306.

Lavilla, E. O. (1987). La larva de *Rhinoderma darwinii* D. & B. (Anura: Rhinodermatidae). *Acta Zoológica Lilloana* **39,** 81-88.

Lavilla, E. O. (1991). Condrocráneo y esqueleto visceral en larvas de *Cycloramphus stejnegeri* (Leptodactylidae). *Amphibia-Reptilia* **12,** 33-38.

Lavilla, E. O. & De Sá, R. O. (2001). Chondrocranium and visceral skeleton of *Atelopus tricolor* and *Atelophryniscus chrysophorus* tadpoles (Anura, Bufonidae). *Amphibia-Reptilia* **22**, 167-177.

Lavilla, E. O. & De Sá, R. O. (1999). Estructura del condrocráneo y esqueleto visceral de larvas de *Pseudis minuta* (Anura: Pseudidae). *Alytes* **16**, 139-147.

Lavilla, E. O. & Vaira, M. (1997). Larval chondrocranium of *Gastrotheca christiani* (Hylidae), with comments on the chondrocrania of non-feeding larvae in Anura. In *Abstracts of the Third World Congress of Herpetology*, 124. Zbynek, R. & Hart, S. (Eds). Prague.

Monteiro, L. R. & Abe, A. S. (1997). Allometry and morphological integration in the skull of *Tupinambis merianae* (Lacertilia: Teiidae). *Amphibia-Reptilia* **18**, 397-405.

Monteiro, L. R. & Abe, A. S. (1999). Functional and historical determinants of shape in the scapula of xenarthran mammals: evolution of a complex morphological structure. *Journal of Morphology* **241**, 251-263.

Monteiro, L. R. & Furtado dos Reis, S. (1999). *Princípios de morfometria geométrica.* Brasil: Holos Editora Ltda-Me.

Monteiro, L. R., Lessa, L. G. & Abe, A. S. (1999). Ontogenetic variation in skull shape of *Trichomys apereoides* (Rodentia: Echinyidae). *Journal of Mammalogy* **80**, 102-111.

Noble, G. K. (1929). The adaptive modifications of the arboreal tadpoles of *Hoplophryne* and the torrent tadpoles of *Staurois*. *Bulletin of the American Museum of Natural History* **58,** 291-337.

Palavecino, P. (1999). Musculatura asociada al primer y segundo arco visceral de algunos anuros leptodactílidos. *Cuadernos de Herpetología* **13**, 37-46.

Petranka, J. W. & Kennedy, C. A. (1999). Pond tadpoles with generalised morphology: is it time to reconsider their functional roles in aquatic communities? *Oecologia* **120**, 621-631.

Rada, D. & Bello, R. (1988). Morfología e histología del sistema digestivo de la larva de *Pseudis paradoxa caribensis* Gallardo, 1961 (Amphibia, Pseudidae). *Iheringia Série Miscellanea* **2,** 69-91.

Ridewood, W. G. (1898). On the larval hyobranchial skeleton of the anurous batrachians, with special reference to the axial parts. *Journal of the Linnaean Society of London* **26**, 474-487.

Rohlf, F. J. (1993). Relative-warp analysis and an example of its application to mosquito wings. *In Contributions to morphometrics*, 131-159. Marcus, L. F., Bello, E., García.Valdecasas, A. (Eds). Madrid: Museo Nacional de Ciencias Naturales.

Rohlf, F. J. (2003). tpsRelw, Version 1.37 Copyright © 2003. New York: Ecology & Evolution, SUNY at Stony Brook.

Rohlf, F. J. (2004). tpsDig, Version 1.40 Copyright © 2004. New York: Ecology & Evolution, SUNY at Stony Brook.

Rohlf, F. J. & Bookstein, F. L. (1990). *Proceedings of the Michigan morphometrics workshop.* Michigan: University of Michigan, Museum of Zoology, Special Publications.

Ruibal, R. & Thomas, E. (1988). The obligate carnivorous larvae of the frog *Lepidobatrachus laevis* (Leptodactylidae). *Copeia* **1988**, 591-604.

Satel, S. & Wassersug, R. J. (1981). On the relative sizes of buccal floor depressor and elevator musculature in tadpoles. *Copeia* **1981**, 129-137.

Seale, D. B. & Wassersug, R. J. (1979). Suspension feeding dynamics of anuran larvae related to their functional morphology. *Oecologia* **39**, 259-272.

Sokol, O. M. (1962). The tadpole of *Hymenochirus boettgeri*. *Copeia* **1962**, 272-284.

SPSS for Windows. (1998). Release 9.0.0. Copyright © SPSS Inc. 1989-1999.

Stayton, C. T. (2005). Morphological evolution of the lizard skull: a geometric morphometrics survey. *Journal of Morphology* **263,** 47-59.

Starrett, P. H. (1960). Description of tadpoles of Middle America frogs. Miscellaneous Publication of the Museum of Zoology, *University of Michigan* **110,** 1-37.

Swart, C. C. & De Sá, R. O. (1999). The chondrocranium of the Mexican burrowing toad, *Rhinophrynus dorsalis* (Anura: Rhinophrynidae). *Journal of Herpetology* **33**, 23-28.

Vera Candioti, M. F. (2005). Morphology and feeding in tadpoles of *Ceratophrys cranwelli* (Anura: Leptodactylidae). *Acta Zoologica* **86**, 1-11

Vera Candioti, M. F. & Haas, A. (2004). Three dimensional reconstruction of the hyobranchial apparatus of *Hyla nana* tadpoles (Anura: Hylidae). *Cuadernos de Herpetología* **18,** 3-15.

Vera Candioti, M. F., Lavilla, E. O. & Echeverría, D. D. (2004). Feeding mechanisms in two treefrogs, *Hyla nana* and *Scinax nasicus* (Anura: Hylidae). *Journal of Morphology* **261**, 206-224.

Vera Candioti, M. F., Úbeda, C. & Lavilla, E. O. (2005). Morphology and metamorphosis of *Eupsophus calcaratus* tadpoles (Anura: Leptodactylidae). *Journal of Morphology* **264**, 161-177.

Wassersug, R. J. (1972). The mechanism of ultraplanktonic entrapment in anuran larvae. *Journal of Morphology* **137**, 279-288.

Wassersug, R. J. (1976). A procedure for differential staining of cartilage and bone in whole formalin fixed vertebrates. *Stain Technology* **51**, 131-134.

Wassersug, R. J. (1980). Internal oral features of larvae from eight anuran families. Functional, systematics, evolutionary and ecological considerations. University of Kansas *Museum of Natural History Miscellaneous Publications* **65,** 1-146.

Wassersug, R. J. (1984). The *Pseudohemisus* tadpole: a morphological link between microhylid (Orton Type 2) and Ranoid (Orton Type 4) larvae. *Herpetologica* **40**, 138-149.

Wassersug, R. J. (1989). What, if anything, is a microhylid (Orton Type II) tadpole? *Fortschritte der Zoologie* **35**, 534-538.

Wassersug, R. J. & Hoff, K. (1979). A comparative study of the buccal pumping mechanism of tadpoles. *Biological Journal of the Linnean Society of London* **12**, 225-259.

Wassersug, R. J. & Pyburn, W. F. (1987). The biology of the Pe-ret' toad, *Otophryne robusta* (Microhylidae), with special consideration of its fossorial larva and systematic relationships. *Zoological Journal of the Linnean Society of London* **91**, 137-169.

Wild, E. R. (1997). Description of the adult skeleton and developmental osteology of the hyperossified Horned frog, *Ceratophrys cornuta* (Anura: Leptodactylidae). *Journal of Morphology* **232,** 169-206.

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APPENDIX 1. Species examined.

Species	Family	Procedence	N	Stage
Bufo arenarum	Bufonidae	Salta, Argentina. December 2003	3	36
Bufo spinulosus	Bufonidae	Jujuy, Argentina. November 2001	3	34
Ceratophrys cranwelli	Leptodactylidae	Salta, Argentina. January 1996	4	33
Chiasmocleis panamensis	Microhylidae	Gamboa, Panamá. July-August 2001	3	35
Elachistocleis bicolor	Microhylidae	Santa Fe, Argentina. January-February 2001	4	34, 35
Hyla microcephala	Hylidae	Gamboa, Panamá. July-August 2001	2	25, 28
Hyla nana	Hylidae	Santa Fe, Argentina. January-February 2001	5	33, 35
Hyla rosenbergi	Hylidae	Gamboa, Panamá. July-August 2001	2	33, 34
Lepidobatrachus llanensis	Leptodactylidae	Salta, Argentina. November 1996	5	33
Lysapsus limellus	Hylidae	Santa Fe, Argentina. January-February 2001	3	31, 35
Phyllomedusa hypochondrialis	Hylidae	Formosa, Argentina. January 2004	3	31, 33, 34
Physalaemus santafecinus	Leptodactylidae	Santa Fe, Argentina. January-February 2001	4	32, 33
Scinax nasicus	Hylidae	Santa Fe, Argentina. January-February 2001	3	32, 35
Telmatobius cf. *atacamensis*	Leptodactylidae	Salta, Argentina. November 2003	2	26, 34

APPENDIX 2. Species from bibliography (categorised according to Altig and Johnston, 1989).

Guild	Species	Family	Reference
ENDOTROPHIC TADPOLES			
Paraviviparous	*Gastrotheca christiani*	Hylidae	Lavilla & Vaira, 1997
Exoviviparous	*Rhinoderma darwinii*	Rhinodermatidae	Lavilla, 1987
Nidicolous	*Eupsophus calcaratus*	Leptodactylidae	Vera Candioti *et al.*, 2005
	Cycloramphus stejnegeri	Leptodactylidae	Lavilla, 1991
	Flectonotus goeldii	Hylidae	Haas, 1996*a*
EXOTROPHIC TADPOLES			
Lotic			
Neustonic	*Phasmahyla guttata*	Hylidae	Fabrezi & Lavilla, 1992
Gastromizophorous	*Atelopus tricolor*	Bufonidae	Lavilla & De Sá, 2001
Clasping	*Atelophryniscus chrysophorus*	Bufonidae	Lavilla & De Sá, 2001
Suctorial. Type I	*Boophis* sp.	Mantellidae	Haas & Richards, 1998
Suctorial. Type II	*Ascaphus truei*	Ascaphidae	Haas, 1996b
	Heleophryne natalensis	Heleophrynidae	Wassersug & Hoff, 1979
	Nyctimystes dayi	Hylidae	Haas & Richards, 1998
Psammonic	*Litoria nannotis*	Hylidae	Haas & Richards, 1998
	Otophryne pyburni	Microhylidae	Wassersug & Pyburn, 1987
Fossorial. Type II	*Leptobrachium hasselti*	Megophryidae	Ridewood, 1898
Lentic			
Arboreal. Type I	*Hoplophryne rogersi*	Microhylidae	Noble, 1929
Arboreal. Type II	*Anotheca spinosa*	Hylidae	Wassersug & Hoff, 1979
Arboreal. Type ?	*Dendrobates tinctorius*	Dendrobatidae	Haas, 1995
Benthic?	*Scaphiopus intermontanus*	Scaphiopodidae	Hall & Larsen, 1998
Suspension-Rasper	*Phyllomedusa boliviana*	Hylidae	Fabrezi & Lavilla, 1992
Suspension-Feeder. Type I	*Xenopus laevis*	Pipidae	Wassersug & Hoff, 1979
Suspension-Feeder. Type II	*Gastrophryne carolinensis*	Microhylidae	Wassersug & Hoff, 1979
Carnivore. Type I	*Ceratophrys cornuta*	Leptodactylidae	Wild, 1997
	Spea bombifrons	Scaphiopodidae	Wassersug & Hoff, 1979
	Leptodactylus pentadactylus	Leptodactylidae	Larson & De Sá, 1998
Carnivore. Type II	*Lepidobatrachus laevis*	Leptodactylidae	Ruibal & Thomas, 1988
Carnivore. Type III	*Hymenochirus boettgeri*	Pipidae	Wassersug & Hoff, 1979
Macrophagous. Type I	*Occidozyga laevis*	Ranidae	Ridewood, 1898
Nectonic. Type I	*Scinax acuminatus*	Hylidae	Fabrezi & Lavilla, 1992
	Pseudis paradoxa	Hylidae	Haas, 2003
	Pseudis minuta	Hylidae	Lavilla & De Sá, 1999
Nectonic. Type II	*Phrynohyas venulosa*	Hylidae	Fabrezi & Vera, 1997

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ECOLOGICAL FUNCTIONS OF THE FOAM NESTS OF THE JAPANESE TREEFROG, *RHACOPHORUS ARBOREUS* (AMPHIBIA, RHACOPHORIDAE)

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Using both field observations and laboratory experiments, thermal and nutritional functions of the arboreal foam nests of the Japanese treefrog *Rhacophorus arboreus* were examined in early summer in the years 2000–2002. The temperature at the centre of the nests and ambient air temperature were monitored using a data logger in the field for several days. The results showed thermal regulation by the foam mass; the inside of the nests was maintained up to 6°C cooler by the foam masses when the ambient temperature was high (>25°C). Laboratory experiments also showed that hatching success of the embryos was very low at high temperatures (near 30°C). The insulation effect of the foam nests is, therefore, considered to be adaptive for *R. arboreus*. Hatchling growth was examined for a week under different food conditions: water only (no food), foam mass and boiled lettuce. Larvae showed no significant growth without food, but they grew to be 2–5 times heavier in dry body mass than hatchlings when supplied with foam mass or boiled lettuce. Foam mass proved to be at least as effective as boiled lettuce as a food for hatchlings. The present study demonstrates the thermal and nutritional effects of the foam nests of *R. arboreus*.

Key words: Amphibia, hatching success, nutritional effect, thermal advantage,

INTRODUCTION

Anuran amphibians exhibit diverse patterns of breeding, and one adaptation to both aquatic and terrestrial breeding is the construction of foam nests in which eggs are deposited in a mass of dense fluid that is whipped into foam by the frogs. Foam-nesting has evolved several times and occurs in at least six families: Rhacophoridae, Leptodactylidae, Myobatrachidae, Hylidae, Microhylidae, and Hyperoliidae (Duellman & Trueb, 1994; Seymour & Loveridge, 1994). A number of possible functions of foam nests have been suggested from the point of view of ecological and adaptive significance: (1) prevention of desiccation of eggs and larvae (Dobkin & Gettinger, 1985; Downie, 1988, 1993); (2) protection against egg predation (Heyer, 1969; Downie, 1988, 1990); (3) thermal advantage (Gorzula, 1977; Dobkin & Gettinger, 1985); (4) potential food resources for hatchlings (Tanaka & Nishihira, 1987); and (5) gas exchange (Seymour & Loveridge, 1994).

The Japanese treefrog, *Rhacophorus arboreus*, is a medium-sized rhacophorid species, usually 50–80 mm in snout-vent length, and distributed across the Honshu and Sado Islands, Japan. It lives in a variety of habitats at elevations from sea level to over 2000 m, but is more abundant in mountain regions. It breeds in early summer, chiefly in ponds surrounded by forested areas (Maeda & Matsui, 1999). Since this species exhibits interesting breeding habits and mating system, e.g. arboreal spawning and multi-male breeding, many researchers have studied its breeding ecology and mating behaviour (e.g., Kato, 1955, 1956; Toda, 1989; Kasuya *et al.*, 1996; Kusano, 1998). Female *R. arboreus* make foam nests attached to branches or leaves of trees along the shore of still waters. We monitored temperatures within and outside the nests for several days using a data logger, and conducted rearing experiments to examine the effect of foam masses on hatchling growth. In the present paper, we report on the thermal and nutritional effects of the foam nests on the embryos and hatchlings of *R. arboreus*, and discuss the ecological functions of the foam nests.

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MATERIALS AND METHODS

STUDY SITE

This study was carried out at a secondary forest, consisting chiefly of *Quercus serrata*, *Q. myrsinaefolia* and *Q. glauca*, within the campus of the Tokyo Metropolitan University (35°37'N, 139°23'E) in Hachioji, Tokyo from May through July of 2000–2002. The forest is approximately 10 ha in area, and contains a small natural pond ("Imori" pond; 10 m diameter, 0.5 m maximal depth) on its western side, at an altitude of 120 m. Since the pond was covered by the canopy of surrounding trees, the shore of the pond was shady during most of the daytime. The breeding population of *R. arboreus* constructed a total of 14–21 foam nests at the shore of the pond every year (2000–2002). This pond is used not only by *R. arboreus* but also by several other amphibian species for breeding: *Bufo japonicus formosus*, *Rana ornativentris*, *R. rugosa*, *Rhacophorus schlegelii*, *Cynops pyrrhogaster* and *Hynobius tokyoensis*.

The climate of the study site is relatively mild, and yearly mean air temperature and yearly rainfall were, on average, 14.3°C and 1563 mm during 1983–2000 at the nearest weather station of the Automated Meteoro-

logical Data Acquisition System (AMEDAS), which is located 8 km north-west of the study site, 123 m in altitude (Japan Meteorological Agency, 2005).

THERMAL EFFECTS OF FOAM NESTS

We monitored long-term fluctuations of temperature within foam nests in the field using a data logger (SK Sato SK-L200T) during the breeding seasons of 2001–2002. We collected a newly constructed foam nest, and attached it to a small branch (about 1–1.5 m above the ground) near the shore of the pond. An electronic thermistor probe (20 mm in length, 5 mm in diameter) connected to the data logger was inserted into the centre of the foam nest, and another probe was placed 1 cm away from the surface of the nest to monitor the ambient air temperature. The temperature was measured to the nearest 0.1°C at intervals of 15 min for 1–7 days. We monitored nest and air temperatures for eight foam nests. Air temperature was also monitored 1.5 m above the ground at the shore of the pond throughout the breeding seasons.

In order to ascertain the effects of temperature on developing embryos of *R. arboreus*, we monitored embryonic development under different temperature regimes, and examined the relationship between temperature and hatching success. We collected a foam nest just after it had been constructed in the pond. Sixty eggs were collected from the nest and divided randomly into three groups (20 per group). The eggs of each group were placed in a Petri dish (15 cm in diameter), which was filled with 400 ml of distilled water, together with a small amount of foam mass. The eggs were removed from the foam and sunk into the water. The three groups were kept at different constant temperatures (13, 21 and 29°C). Developing embryos were inspected every day. Halted or impaired development in the embryos was indicated by malformation, discoloration, fungal infestation or any combination of the above factors, and we counted dead embryos and hatchlings. When dead embryos and hatchlings were found in the petri dishes, they were removed.

Since foam nests had, on average, 533 eggs (SD=133, n=24) in the study pond, many eggs remained within the nests even after 60 eggs were taken. The foam nest used in the experiment was therefore returned to the pond and attached to small branches on the shore. A large bucket (volume 20 l) filled with pond water was placed beneath the nest to collect hatchlings dropping down from the nest. When hatchlings began to emerge, the nest was collected again, and dead embryos and hatchlings were counted to determine hatching success in the field. We repeated the procedures for a total of five nests during the breeding season of 2001.

NUTRITIONAL EFFECTS OF THE FOAM

We visited the study pond at least once a day during the breeding season of *R. arboreus*, and searched for newly constructed foam nests. When such nests were found, their positions were recorded on a map and monitored until the hatchlings emerged from the nests. From those nests constructed in 2000, we collected a total of four nests just after the hatchlings began to emerge from the nests. From each nest, 30 hatchlings (stage 22; Gosner, 1960) were collected randomly, and their total lengths and snout-vent lengths (SVL) were measured to the nearest 0.1 mm with a vernier calliper. They were divided into three treatment groups (10 hatchlings per group). Hatchlings of each group were placed in a water bath (17 cm x 27 cm x 17 cm), which was filled with 5 l of tap water purified by water filter. Different nutritional conditions were assigned to three treatment groups: (1) water only (no food); (2) an *ad libitum* amount of foam mass that was taken from their nest; and (3) an *ad libitum* amount of boiled lettuce. Hatchlings were reared at a constant temperature of 23±0.5°C with 14L:10D photoperiod for one week. Water in the baths was not changed until the end of the experiment. After the experiment, all tadpoles were anaesthetized and killed with MS222, their total lengths and SVLs were measured, and the developmental stages were recorded according to Gosner (1960). Dry body masses of individual tadpoles were determined to the nearest 0.01 mg by an electronic reading balance (Mettler AT201) after they had been dried at 55°C for 24 hours.

In order to determine the initial body masses of hatchlings, 10 additional hatchlings were also collected at random from each nest, and their dry masses were determined in the same way as described above.

STATISTICAL ANALYSIS

Statistical analyses were performed with the Statistical Analyses Software version 6.12 (SAS Institute Inc., 1990). Difference in temperatures inside and outside the foam nests was tested using paired t-tests for each nest examined. The effects of temperature and nests on hatching success were examined using two-way analysis of variance (ANOVA) without replication, after the rate of the number of hatchlings to the total number of eggs (hatching success) was arcsine transformed. Pairwise comparisons were also conducted between different temperatures following the procedure of the Tukey test (Zar, 1996). The significance level in all tests was $P<0.05$.

For each nest, pairwise comparisons of dry body masses of larvae at the end of the experiment were conducted between nutritional conditions using Welch's test, because of heterogeneity of variances (Zar, 1996). Acceptance levels for simultaneous statistical tests were adjusted by the sequential Bonferroni procedure (Rice, 1989). Results where the adjusted P-value was less than 0.05 were judged statistically significant.

RESULTS

THERMAL EFFECTS OF THE FOAM NESTS

The temperatures inside and outside the nests were monitored concurrently for eight nests using a data log-

TABLE 1. Data on air temperature at the study site during the breeding season of *R. arboreus*. Data on monthly rainfall obtained at the nearest AMEDAS weather station (8 km north-west of the study site) is also shown (Japan Meteorological Agency, 2005).

	Air temperature (°C)			
Month	Mean	Min	Max	Rainfall (mm)
2001				
May	17.2	6.5	25.5	158.1
June	20.5	14.9	28.8	76.3
July	25.9	19.8	33.9	231.7
2002				
May	15.8	9.7	26.1	130.9
June	18.9	13.0	28.4	100.1
July	25.1	19.9	31.2	141.4

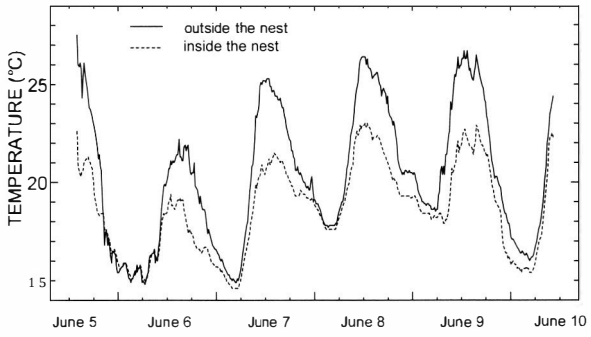

FIG. 1. Daily fluctuations of temperature inside a foam nest (No.12) during June 5–10 in 2002 (dashed line). Air temperature just above the outer surface of the nest is shown by the solid line.

ger; each nest was monitored for, on average, 4.4 days (SD=1.7, Table 2). We also monitored air temperature at the shore of the pond, and Table 1 shows climatic conditions of the pond during the study period.

Fig. 1 shows diel changes of both temperatures for nest No.12. From the middle of the night to early the next morning, the nest temperature was almost equal to the ambient air temperature, but as the air temperature rose gradually, the difference in temperature increased. When the air temperature rose above 25°C in the afternoon, the nest temperature was maintained at about 3--4°C lower than the ambient air temperature. For most of the other nests, similar relationships were observed between nest and air temperatures. The mean temperature was significantly lower inside the nests than outside in almost all nests (six out of eight), although the opposite result was observed in nest No.2 in 2001 (Table 2). At present, we do not know the reasons for this result in nest No.2.

When the air temperature was low (<20°C), the difference in temperature inside and outside the nests was comparatively small. As the air temperature rose gradually, the difference in temperature increased, because the temperature inside the nests did not rise at the same rate as the air temperature (Fig. 2). At air temperatures higher than 25°C, the difference in temperature between the centre of foam nests and the ambient air reached a maximum of 6°C. This result demonstrates thermal

TABLE 2. Mean air and nest temperatures in the foam nests of *R. arboreus*. Air temperatures just above the outer surface of the nest and nest temperatures at the centre of the foam nests were monitored at intervals of 15 minutes using a data logger.

		Temperature (°C) mean±SD (range)		Paired *t*-test	
Nest	Period	air	nest	*n*	*P*
2001					
No.1	May 17–21	19.00±3.94 (11.9-28.4)	17.85±2.84 (12.1-24.6)	395	<0.0001
No.2	May 21–25	18.3±1.42 (16.0-23.4)	18.52±1.46 (16.3-24.0)	386	<0.0001
No.9	May 29–30	17.84±1.62 (14.1-22.2)	17.64±2.22 (15.1-20.4)	99	0.072
No.10	May 25–29	18.68±2.48 (13.9-26.30)	18.22±1.80 (14.4-22.6)	369	<0.0001
2002					
No.2	May 16–20	13.99±2.31 (11.3-22.6)	13.37±2.00 (10.8-19.2)	381	<0.0001
No.7	May 30–Jun 6	20.33±2.83 (15.9-27.7)	19.48±2.55 (15.7-28.2)	572	<0.0001
No.12	Jun 5–10	20.36±3.45 (14.8-27.5)	18.64±2.31 (14.6-23.0)	467	<0.0001
No.17	Jun 21–28	16.45±2.10 (14.2-25.3)	16.08±1.70 (14.1-22.3)	669	<0.0001

regulation by the foam mass; the inside of nests was maintained cooler to some extent by the foam masses when the ambient temperature was high.

Embryos kept at different temperatures required from three days to three weeks to hatch (stage 22). Many dead embryos halted their developments before stage 10 (gastrulation) without fungal infection at 13°C, but most dead embryos reached later developmental stages and showed fungal infection at higher temperatures, especially at 29°C. Hatching success varied greatly from 0 to 0.8 under constant temperatures, and from 0.51 to 0.91 in the field (Fig. 3). Effects of temperature and nest on hatching success were examined using two-way ANOVA after arcsine transformation, and the results showed a significant effect of temperature ($F_{3,12}$=17.54, P<0.0001) as well as nest ($F_{4,12}$=5.17, P=0.0118). Hatching success was significantly lower at 13 and 29°C than in the field, but the difference between 21°C and field conditions was not significant. This result shows that the embryos of *R. arboreus* were vulnerable to both low and high temperatures, and high temperatures (near 30°C) in particular were lethal to the embryos.

NUTRITIONAL EFFECTS OF THE FOAM

Four nests (Nos. 2, 3, 5, and 8) were used for the experiment on hatchling growth under different nutritional conditions. Although six larvae from nest No.2 died during the experiment, all the larvae from the other three nests survived until the end of the experiment. By the end of the experiment, hatchlings (stage 22) had reached different stages for different food conditions (Kruskal-Wallis test, χ^2=22.71, P<0.001): the median stage was 26 (range 25–28) for no food, 28 (26–28) for foam, and 28 (26–29) for lettuce. The developmental rate of

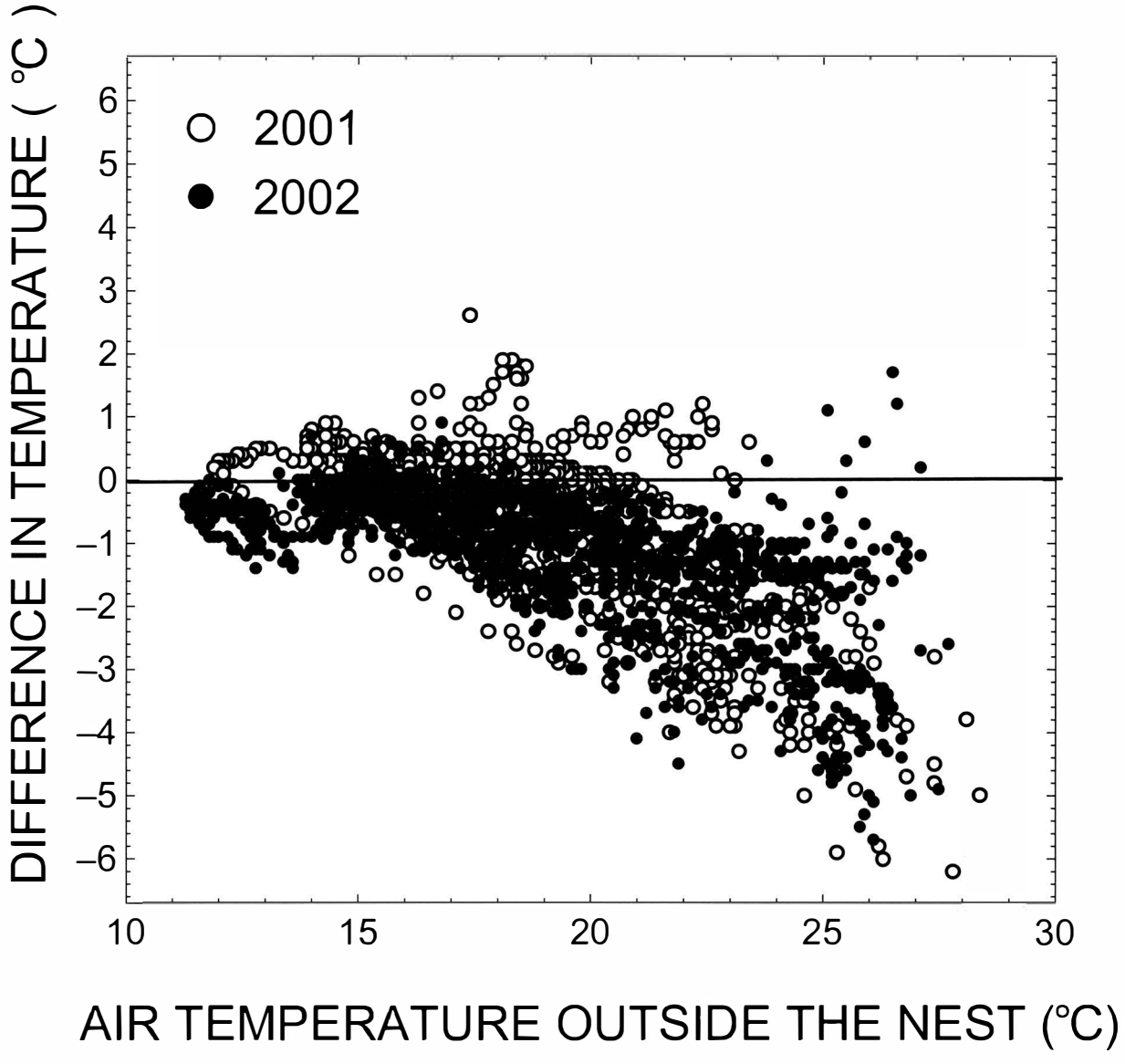

FIG. 2. Relationship between nest and air temperatures. Nest and air temperatures were measured at an interval of 15 min at the centre of the foam nests and 1 cm away from the surface of the nests, respectively. Differences in temperature (nest minus air) are plotted against air temperature. Data from a total of eight nests for 35 days were plotted (n=3338).

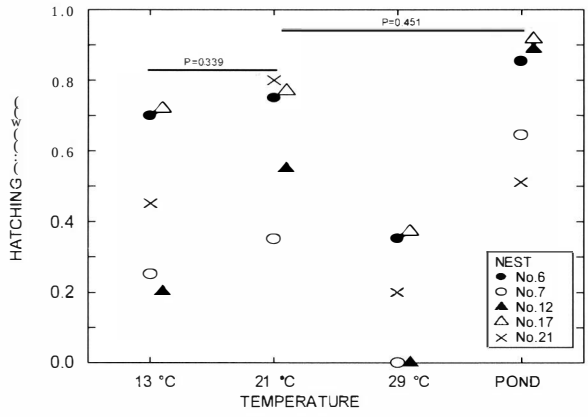

FIG. 3. Effects of temperature on hatching success of the embryos. Embryos from five nests were kept at three different constant temperatures (13, 21, and 29°C) and under field (pond) conditions. Mean air temperatures during the field incubation periods were 19.1 (nest No.6), 18.8 (No.7), 18.7 (No.12), 19.0 (No.17), and 24.6°C (No.21), respectively. Horizontal bars indicate non-significant differences ($P>0.05$) between temperatures (Tukey's test after arcsine transformation).

hatchlings was significantly reduced when no food was supplied (Newman, 1998), but development was similar in the foam and lettuce food conditions.

Dry body masses of hatchlings and larvae at the end of the experiment are shown in Fig. 4. Mean body masses of hatchlings varied slightly from 4.28 to 4.76 mg among the nests, and mean body masses of larvae at the end of the experiment varied markedly from 4.49 to 26.29 mg among treatment groups. Pairwise comparisons were made between different food conditions within the nests. Larvae showed no significant growth without food, but they grew to be 2–5 times heavier than hatchlings when supplied with foam mass or boiled lettuce as food (Fig. 4). Larval growth when foam mass was supplied was equal to, or even higher than, that when boiled lettuce was supplied. Mean body mass of the larvae supplied with foam mass was more than twice that of the larvae supplied with lettuce for nest No.8. The effect of food conditions on growth in SVL was quite similar to that on growth in dry body mass. These results show that foam mass of *R. arboreus*, as well as boiled lettuce, is an effective food resource for hatchlings.

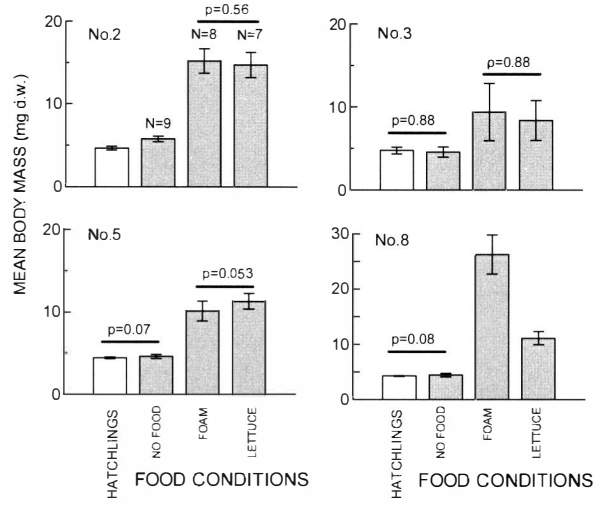

FIG. 4. Growth of hatchlings under different nutritional conditions. Hatchlings were taken from four nests, and reared at a constant temperature (23°C) for a week. Grey and clear histograms indicate mean dry body masses of larvae at the end of the experiment and those just after hatching, respectively. Vertical bars indicate 1 SD. Sample sizes (N) are all 10 except for where N is explicitly indicated (data on six dead larvae of nest No.2 were excluded from analysis). Horizontal bars indicate non-significant differences, but other comparisons are all significant ($P<0.05$, Welch's test, sequential Bonferroni adjustment).

DISCUSSION

Temperature Regulation

The thermal advantage of foam nests has been examined by a number of researchers, but their results are not consistent with each other. Gorzula (1977) measured the temperature of foam nests of *Physalaemus enesefae*, and found that nest temperature was almost 5°C less than that of adjacent water. He suggested that foam keeps eggs cool to prevent thermal damage. However, Dobkin & Gettinger (1985) measured the temperature of foam nests of *P. pustulosus* and obtained the opposite result. Absorption of solar radiation by nests resulted in temperatures up to 8.2°C above ambient air temperature, allowing more rapid development. Downie (1988) examined the thermal properties of foam nests more completely than the previous authors, using *P. pustulosus*, and his data supported neither study. Foam temperature was very similar to ambient water or air temperatures, and time to hatching did not differ whether in foam or floating on water. No thermal effect of the foam was detected.

We monitored long-term temperature fluctuations of arboreal foam nests of *R. arboreus* using a data logger. The observation lasted for a total of 35 days using eight foam nests. Our data on the fluctuation of temperatures inside and outside the nests shows a significant thermal effect of the foam (Table 2 and Fig. 2). The inside of nests was maintained up to 6°C lower by the foam masses when the ambient temperature was high, which suggests that temperature is regulated by the foam. The white foam of nests may reflect solar radiation, and evaporation from the foam may cool down egg temperatures (but see Dobkin & Gettinger, 1985). The temperature regulation is, however, considered to be chiefly due to the insulation effect of the foam masses. Our result is consistent with that of Gorzula (1977). There may well be differences in thermal properties between foam nests which float on water and those surrounded by air and attached to branches.

The effect of temperature on hatching success was examined, and we detected a significant effect. Both high and low temperatures had detrimental effects on embryos, and especially at high temperatures (near

30°C), most of the embryos did not survive to hatching (Fig. 3). If the insulation effect of the foam prevents the nests from overheating (near 30°C), foam nests must be adaptive for *R. arboreus*, since embryonic mortality is kept low.

Foam as a Potential Nutritional Resource

Foam could be a food source for hatchlings, either directly or by offering a substrate for microorganisms (Downie, 1988). Tanaka & Nishihira (1987) examined this possibility using *R. viridis*, which makes terrestrial foam nests at pond edges, and demonstrated that the foam mass can promote hatchling growth effectively. The present study showed a similar result in *R. arboreus* (Fig. 4). At present, however, we do not know what produces the nutrient effect: the foam itself or microorganisms involved in foam breakdown.

A chemical analysis of the freshly produced foam of *Polypedates leucomystax* showed that dried foam substances consist of protein (93.7%) and anthronpositive sugars (6.3%), and that among the 17 amino acids detected, asparagine, lysine and glutamate are relatively abundant (Kabisch *et al.*, 1998). Promotion of hatchling growth by foam mass might be attributable to these components, although the chemical composition of the foam mass of *R. arboreus* is not yet known.

One or two weeks after nest construction, foam nests with hatchlings dissolve by themselves when they are exposed to high humidity or rain drops. Most of the hatchlings that escape from foam nests still have yolk supplies in the abdomen. However, hatchlings may not be able to escape from the nests when the outer layers of foam nests become dry and hardened during droughts (e.g. Downie, 1993). It is possible that in such situations, hatchlings use the foam masses as their food sources within the nests (Tanaka & Nishihira, 1987). In addition, the tadpoles of *R. arboreus* were observed to gather around the foam masses that had dropped down on the water surface to feed on the foam (personal observation). Females of *R. arboreus* sometimes construct foam nests above small temporary water bodies, such as side ditches or wheel ruts in forest roads (personal observation). Foam masses may be an effective nutritional resource for tadpoles, especially in such small water bodies. The foam masses can, therefore, be a potential food resource for hatchlings both inside and outside the nests.

Other Functions

Two of five suggested functions of foam nests were examined in detail, as mentioned above. The remaining functions may be also effective in *R. arboreus*: prevention of desiccation of eggs and larvae (Dobkin & Gettinger, 1985; Downie, 1988, 1993), protection against egg predation (Heyer, 1969; Magnusson and Hero, 1991; Downie, 1988, 1990) and gas exchange (Seymour & Loveridge, 1994). Of these suggested functions, prevention of desiccation is the most likely to be an actual function of the foam nests of *R. arboreus*.

In *R. arboreus*, arboreal spawning occurs. If foam nests are not constructed and eggs are deposited directly on leaves or branches of trees without foam mass, the eggs would surely dry up quickly. Hence, it is highly likely that the foam must prevent eggs from becoming desiccated.

In a laboratory experiment using foam nests of *P. pustulosus*, Downie (1990) showed that a much higher proportion of floating eggs presented as individuals were predated by *Leptodactylus fuscus* tadpoles than as groups embedded in foam, and demonstrated that *P. pustulosus* eggs are somewhat protected from predation by being surrounded in foam. In the population studied, the most important predator of eggs was a snake, *Amphiesma vibakari*, which was sometimes observed to eat eggs by crashing the head into the foam nests (personal observation). Foam nests are not effective as a protective device against large vertebrate predators such as snakes, but may be effective against predation by small invertebrates. We observed in detail a total of 24 foam nests during the 2000–2002 breeding seasons, and only one of them was attacked by invertebrate predators: dipteran larvae (not identified, see Vonesh, 2000). This fact may indicate significant anti-predator effects of foam nests, but we do not have any definite evidence at present.

The foam of terrestrial nests may also be advantageous, as it allows the depositing of a clutch much larger than would be possible were the eggs surrounded by jelly alone. The bubbles in the foam not only facilitate oxygen diffusion into the egg mass but also provide a capacious oxygen store for immediate use by embryos, without the need for diffusion over long distances (Seymour & Loveridge, 1994). Female *R. arboreus* construct a large spherical foam nest, 10–15 cm in diameter, including 300–800 eggs above pond waters (Maeda & Matsui, 1999). If the respiratory role of the foam is not effective, production of such a large clutch may be impossible (see Seymour & Loveridge, 1994). But at present we have no data on the respiratory role of the foam in *R. arboreus*, and further studies are needed to investigate this.

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REFERENCES

Dobkin, D. S. & Gettinger, R. D. (1985). Thermal aspects of anuran foam nests. *Journal of Herpetology* **19**, 271-275.

Downie, J. R. (1988). Functions of the foam in the foam-nesting Leptodactylid *Physalaemus pustulosus*. *Herpetological Journal* **1**, 302-307.

Downie, J. R. (1990). Functions of the foam in foam-nesting Leptodactylids: anti-predator effects of

Physalaemus pustulosus foam. *Herpetological Journal* **1**, 501-503.

Downie, J. R. (1993). Functions of the foam in foam-nesting Leptodactylids: the nest as a post-hatching refuge in *Physalaemus pustulosus*. *Herpetological Journal* **3**, 35-42.

Duellman, W. E. & Trueb, L. (1994). *Biology of Amphibians*. Baltimore: Johns Hopkins University Press.

Gorzula, S. (1977). Foam nesting in Leptodactylids: a possible function. *British Journal of Herpetology* **5**, 657-659.

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

Heyer, W. R. (1969). The adaptive ecology of the species groups of the genus *Leptodactylus* (Amphibia, Leptodactylidae). *Evolution* **23**, 421-428.

Japan Meteorological Agency (2005). Meteorological data reference room. URL http://www.data.kishou.go.jp

Kabisch, K., Herrmann, H.-J., Klossek, P. & Brauer, K. (1998). Foam gland and chemical analysis of the foam of *Polypedates leucomystax* (Gravenhorst 1829) (Anura: Rhacophoridae). *Russian Journal of Herpetology* **5**, 10-14.

Kasuya, E., Hirota, M. & Shigehara, H. (1996). Reproductive behavior of the Japanese treefrog, *Rhacophorus arboreus* (Anura: Rhacophoridae). *Researches on Population Ecology* **38**, 1-10.

Kato, K. (1955). Ecological notes on the green frogs during the breeding season. I. *Japanese Journal of Ecology* **5**, 70-73. (In Japanese, with English summary)

Kato, K. (1956). Ecological notes on the green frogs during the breeding season. II. Breeding habits and others. *Japanese Journal of Ecology* **6**, 57-61. (In Japanese, with English summary)

Kusano, T. (1998). A radio-tracking study of post-breeding dispersal of the treefrog, *Rhacophorus arboreus* (Amphibia: Rhacophoridae). *Japanese Journal of Herpetology* **17**, 98-106.

Maeda, N. & Matsui, M. (1999). *Frogs and Toads of Japan*, 2nd ed. Bun-Ichi Sogo Shuppan, Tokyo. (In Japanese, with English abstract)

Magnusson, W. E. and Hero, J.-M. (1991). Predation and the evolution of complex oviposition behaviour in Amazon rainforest frogs. *Oecologia* **86**, 310-318.

Newman, R. A. (1998). Ecological constraints on amphibian metamorphosis: interactions of temperature and larval density with responses to changing food level. *Oecologia* **115**, 9-16.

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

SAS Institute Inc. (1990). *SAS/STAT User's Guide, Version 6, Vols. 1 and 2*, 4th ed. Cary: SAS Institute Inc.

Seymour, R. S. & Loveridge, J. P. (1994). Embryonic and larval respiration in the arboreal foam nests of the African frog *Chiromantis xerampelina*. *Journal of Experimental Biology* **197**, 31-46.

Tanaka, S. & Nishihira, M. (1987). Foam nest as a potential food source for anuran larvae: a preliminary experiment. *Journal of Ethology* **5**, 86-88.

Toda, M. (1989). *The Life History of Japanese Forest Frog,* Rhacophorus arboreus *in the Kanazawa Castle* (Master's Thesis). Kanazawa: Kanazawa University. (In Japanese)

Vonesh, J. R. (2000). Dipteran predation on the arboreal eggs of four *Hyperolius* frog species in western Uganda. *Copeia* **2000**, 560-566.

Zar, J. H. (1996). *Biostatistical Analysis*, 3rd ed. Upper Saddle River: Prentice-Hall.

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LIFE IN THE WATER: ECOLOGY OF THE JACARERANA LIZARD, *CROCODILURUS AMAZONICUS*

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The semi-aquatic teiid lizard *Crocodilurus amazonicus* (local name jacarerana) inhabits lakes and rivers throughout Amazonia. Although it is a common species in many areas, very little information is available on its biology. I provide information on the ecology of *C. amazonicus* in areas of flooded forests in central Amazonia, Brazil. Most field observations were made at two igapó (blackwater swamp) forest in the Negro River basin, from 1992 to 1995. Lizards were found accidentally or during time-constrained searches by boat or on foot. More than 100 individuals were observed in both areas. Lizards were either swimming in shallow waters or exposed on the ground or on low vegetation. During low water, when large expanses of shoreline became exposed, *C. amazonicus* foraged and basked on these margins. When the water began to rise and several ponds were formed in the igapó forests, the lizards moved into the flooded forest. They were much easier to find during low water. The jacarerana feeds on several prey types, but eats more crustaceans and other aquatic animals than terrestrial teiids. I found 85 prey items in 26 stomachs. Arthropods (insects, shrimps, crabs and spiders) comprised about two thirds of total prey volume and vertebrates (fish and frogs, including tadpoles) about one third. Because most prey were aquatic, *C. amazonicus* probably forages mainly in the water. The jacarerana may be the only Neotropical lizard that feeds frequently on fish (23% of total prey volume) and crabs (16%). The occurrence of *C. amazonicus* in many protected areas in Brazil and adjacent countries may offset population declines associated with development in the future.

Key words: Amazonia, aquatic lizard, piscivory, swamp forest, Teiidae

INTRODUCTION

Teiid lizards are conspicuous elements of Neotropical habitats. Although most species of teiids are terrestrial (mainly those in the speciose genera *Cnemidophorus* and *Ameiva*), species in two genera (*Crocodilurus* and *Dracaena*) are semi-aquatic and at least *Tupinambis teguixin* and four species of *Kentropyx* occasionally enter water (see review in Vitt *et al.*, 2001). Indeed, besides *Crocodilurus* and *Dracaena*, *Kentropyx altamazonica* is closely associated with water courses in Amazonia (Vitt *et al.*, 2001). In the last decade, the ecology of many Amazonian teiid lizards that inhabit forests have been studied in detail (e.g. Vitt *et al.*, 1995, 1997, 2000, 2001; Sartorius *et al.*, 1999). However, the ecology of species that are found almost exclusively along large rivers and lakes, especially *Dracaena* and *Crocodilurus*, is still largely unknown (see review in Ávila-Pires, 1995). Because the water level of these lakes and rivers varies considerably during the year, these lizards should respond to fluctuating shorelines in an observable manner.

The jacarerana, *Crocodilurus amazonicus* (Fig. 1), inhabits the margins of rivers, creeks and lakes in the Amazon basin (Goeldi, 1902; Crump, 1971; Vanzolini, 1972, 1993; Hoogmoed & Lescure, 1975; Gasc, 1990; Ávila-Pires, 1995; Bartlett & Bartlett, 2003) and occurs from Peru eastwards to Belém, Pará, Brazil, and from the Orinoco in Venezuela southwards to the upper Madeira River, Amazonas, Brazil (Ávila-Pires, 1995).

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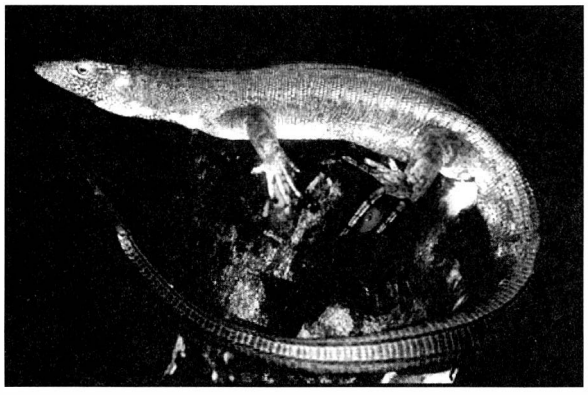

FIG. 1. An adult *Crocodilurus amazonicus* from Tartarugalzinho, Amapá, Brazil (G. R. Colli).

Published data on the feeding habits of *C. amazonicus* are scarce. Vanzolini & Valencia (1965) described its dentition as being of the insectivore pleurodont type. Ávila-Pires (1995), quoting M. Martins, reports on a stomach of an adult from Cuieiras River that contained 10 juvenile *Bufo marinus*, a dragonfly and a hemipteran. Bartlett & Bartlett (2003) stated that *C. amazonicus* feeds on fish, amphibians, insects, worms and spiders, but without supporting data.

Here I provide information on the ecology of populations of *C. amazonicus* from central Amazonia, Brazil.

MATERIALS AND METHODS

Field observations were made at Miratucu Lake (1°57'08"S, 61°49'20"W, elevation approx. 100 m), Jaú River, and Jaradá Lake (2°8'34"S, 60°21'26"W),

Cuieiras River, Amazonas, Brazil; both lakes are large basins of still water connected to blackwater rivers of the Negro River basin. Three field trips were made in 1995 to Miratucu Lake (24 March to 15 April; 7 June to 3 July; 9–29 November). Two field trips were made to Jaradá Lake (12–19 August, 1992; 31 March to 5 April, 1993). Additional observations of *C. amazonicus* were made by other researchers doing fieldwork at Jaradá lake in 1992 and 1993.

Lizards were found accidentally or during time-constrained searches. In both Miratucu and Jaradá lakes, I searched for *C. amazonicus* by boat or on foot along the margins of the lake and of ponds of various sizes and flooded streambeds in the igapó forest.

At Jaú River, 31 lizards were collected by hand, killed by a lethal injection of Nembutal●, measured (snout-vent length and tail length) and weighed. These specimens were deposited in the collection of Instituto Nacional de Pesquisas da Amazônia (INPA) at Manaus, AM, Brazil. Stomach contents were examined under a dissecting microscope and measured (length, width and height) with callipers. Prey volume was calculated with the formula for an ellipsoid ($V=4/3\pi \times$ length $\times$ width $\times$ height). Linear regression analysis was used to test whether lizard body size influences mean and maximum prey volume. To compare the degree of generalization in the diet of *C. amazonicus* with that of other teiids, niche breadth values for numerical and volumetric diet data were calculated as in Vitt *et al.* (2000). Prey categories used herein are similar to those in the studies used to compare the niche breadth of *C. amazonicus* (Howland *et al.*, 1990; Vitt & Colli, 1994; Vitt & Ávila-Pires, 1998; Vitt *et al.*, 2001).

At Cuieiras River, ten Tomahawk mammal traps (six of them approx. 65 × 23 × 23 cm and four 40 × 15 × 15 cm) were left on the ground along the margins of the lake for four days (40 trap-days). Traps were baited with fish and banana (to attract flies). Data gathered during accidental observations made in other areas in Amazonia are also provided.

RESULTS

At Miratucu and Jaradá lakes, about 110 individuals of *C. amazonicus* were observed within the igapó margins, mostly on sunny days. Most observations were made during low water (October through January), when large extensions of unvegetated, leaf-litter-covered margins became exposed. Lizards were either swimming in shallow waters or exposed on the ground or on low vegetation (mainly on slender branches of fallen trees, sometimes over the water). No lizard was observed in areas of deep water. When approached, *C. amazonicus* fled by swimming distances of up to 20 m, most often parallel to the lake or river bank. They also submerged and concealed themselves inside the leaf litter of the bottom or sought retreat in burrows in the margin, mostly under fallen tree trunks; juveniles also sought retreat in hollows inside fallen tree trunks.

During the first trip to Miratucu Lake (24 March to 15 April), the water level was rising and only some narrow stretches of the lake margins were exposed. Several large, shallow ponds were forming within the igapó forests inland. The streambeds inside igapó forests were also becoming flooded. I searched for *C. amazonicus* by boat or on foot through the few exposed margins of the lake and the margins of flooded streambeds and ponds of various sizes inside the igapó forest. Six *C. amazonicus* were observed during 23.5 man-hours of diurnal visual searching (0.25 *C. amazonicus*/man-hour), most of them inside the partially flooded igapó forest (mainly on the margins of large ponds). An additional individual was found at night, apparently asleep within the leaf litter in the shallows of a large pond in the igapó forest, several metres from the flooded lake margins.

During the second field trip to Miratucu Lake (7 June to 3 July), the water level was very high (the peak of flooding) and all the lake margins were flooded. Only the highest portions of the igapó forest (tree crowns) were exposed; I failed to find the lake margins inside the igapó (it became a labyrinth of water and tree trunks). I visually searched for *C. amazonicus* by boat through the flooded lake margins and streambeds. No *C. amazonicus* was found during 41.5 man-hours of diurnal visual searching.

During the third field trip to Miratucu Lake (9–29 November) the water level was very low and large extensions of lake margins were exposed. Fifty-five *C. amazonicus* were observed on this trip, during 76.6 man-hours of visual searching (0.72 *C. amazonicus*/man-hour). Most of these individuals were juveniles 220–300 mm in total length. No *C. amazonicus* was found in the streams that cut through the unflooded igapó forests.

In addition to the Miratucu Lake observations above, additional individuals were seen along the margins of the Jaú River proper on all field trips, although these sightings were not quantified. Most of them were basking either on fallen tree trunks or on rocks, and fled by jumping into the water. Apparently, *C. amazonicus* is far less common along rivers than it is along lake margins.

About 30 *C. amazonicus* were observed along the margins of the Jaradá Lake and the Cuieiras River proper during low waters of 1992 and 1993. Most were either in still or slow-moving waters. Behaviour and microhabitat use by these lizards were identical to those observed at Jaú River (see above). One individual (total length about 50 cm) observed at Jaradá Lake, Cuieiras River, in August 1992, basked on the emerging leafless twigs of a partially submerged tree about 20 m offshore for one hour (1030 hrs to 1130 hrs). It then swam slowly about 60 m towards an emerging tree trunk (diameter ca. 50 cm) about 10 m offshore, where it hid inside a large hollow. The mammal traps failed to catch *C. amazonicus* at the margins of Jaradá Lake; only a small *Ameiva ameiva* was caught with a trapping effort of 40 trap-days.

At the Anavilhanas archipelago (Negro River, north of the mouth of the Cuieiras River), *C. amazonicus* was also found to be common in igapó forests (R. Silveira, pers. comm.). In February 1993, at Janauari Lake, a whitewater lake on the right bank of the Amazonas River, near its confluence with the Negro River, also in central Amazonia, a large *C. amazonicus* (total length ca. 60 cm) was basking by day at the top of a standing dead tree trunk (2 m high) in the middle of a stream (ca. 10 m wide) that cuts through floodable várzea forests. One specimen at Oriximiná, Pará, Brazil (MZUSP 52513), attempted to hide in a hollow log in the middle of a 2 m wide forest stream, 300 m from its confluence with the Trombetas River (M. T. U. Rodrigues, pers. comm.).

Additional lizard species commonly observed on the margins and associated igapó forests of both Jaradá and Miratucu lakes were the teiids *Ameiva ameiva*, *Kentropyx altamazonica* and *Tupinambis teguixin*, and the tropidurid *Uranoscodon superciliosus*. As with *C. amazonicus*, the three teiids were far easier to observe during low rather than high water. *Ameiva ameiva* was found mainly in sunspots inside the igapó forest, while *K. altamazonica* and *T. teguixin* were both relatively common in the lake margins. During high water, *Uranoscodon superciliosus* was very common in the tree trunks of the flooded lake margins, but it was uncommon during low water.

Thirty-one specimens of *C. amazonicus* collected at Jaú River were examined for stomach contents. Five stomachs were empty; a total of 85 prey items were found in the 26 remaining stomachs (Table 1). In general, arthropods (insects, crustaceans and spiders) represented about two thirds (69%) of total prey volume, the remaining one third (31%) being vertebrates (fish and frogs; Table 1). The main food types (with over 5% of total prey volume) were: fish (22.7%), crustaceans (17.9%), hemipterans (17.1%), dipterans (12.2%), coleopterans (12.0%) and frogs (including tadpoles, 8.0%). Hemipterans, coleopterans, fish and spiders occurred in at least 30% of the stomachs (Table 1). As with the vertebrates, almost all invertebrates found in the stomachs of *C. amazonicus* were aquatic. Niche breadths for numerical and volumetric data in Table 1 were 8.1 and 7.5, respectively.

In general, the major food types of juveniles (SVL 74–101 mm, n=20) were similar to those of subadults and adults (SVL 158–218 mm, n=6), although our sample is too small for adults to allow a detailed analysis. Lizard SVL seems to determine mean and maximum volume of the prey ingested by *C. amazonicus* (all variables log transformed; n=23 for both analyses, R^2=0.289 and 0.278, F=8.523 and 8.078, P=0.008 and 0.010, respectively). Furthermore, mean and maximum prey volume increased proportionally to lizard SVL, since the slopes of the regression lines were not significantly different from one (b=2.093 and 2.010, t=1.524 and 1.427, respectively, P>0.05 in both analyses); i.e. there was no ontogenetic shift in the relative volume of prey ingested in *C. amazonicus*.

None of the three adult females (SVL 173, 179 and 222 mm) collected at the Jaú River in November, 1995 were reproductive. However, the largest one apparently had already reproduced (wrinkled oviduct walls), while the smaller ones were apparently non-reproductive (smooth oviduct walls). Five juveniles (SVL 73–88

TABLE 1. Stomach contents (n=85 prey items) of 26 *Crocodilurus amazonicus* from Jaú River, central Amazonia, Brazil. Results for major taxonomic groups are in boldface. Abbreviations are: n=number of prey; stm = number of stomachs in which a given prey type occurred; vol = volume (cm^3) of prey. Anurans include two tadpoles.

Prey type	*n*	stm	vol	% n	% stm	% vol
Crustaceans (total)	5	4	2.69	5.9	15.4	17.9
crab	3	2	2.45	3.5	7.7	16.4
shrimp	2	2	0.24	2.3	7.7	1.5
Insects (total)	51	17	7.27	60.0	65.4	48.8
dipteran	14	1	1.82	16.5	3.8	12.2
coleopteran						
hydrophiliid	5	3	0.41	5.9	11.5	2.7
staphylinid	1	1	0.22	1.2	3.8	1.5
other	5	4	1.17	5.9	15.4	7.8
ephemeropteran	1	1	0.18	1.2	3.8	1.2
hemipteran						
belostomatid	16	9	2.33	18.8	34.6	15.6
other	2	2	0.22	2.3	7.7	1.5
hymenopteran (pupae)	6	1	0.60	7.0	3.8	4.0
orthopteran grillotalpid	1	1	0.32	1.2	3.8	2.1
Spiders	12	8	0.33	14.1	30.1	2.1
Fish	13	8	3.38	15.3	30.1	22.7
Anurans	4	3	1.20	4.7	11.5	8.0

mm) collected in November, 1995 at Jaú River had evident umbilical scars, indicating that they were born in the previous few weeks, at the onset of the low waters.

DISCUSSION

During over seven years of fieldwork around Manaus, central Amazonia (see, for example, Martins & Oliveira, 1998), *C. amazonicus* was never observed in streams that cut terra firme (=non-floodable) forests, where the gymnophthalmid *Neusticurus bicarinatus* and the tropidurid *Uranoscodon superciliosus* were always common (pers. obs.). Thus, at least in central Amazonia, *C. amazonicus* is apparently always associated with streams, rivers and lakes in areas of igapó and várzea forests. Furthermore, it seems to be restricted to areas of still or slow moving water.

The variation of water level in igapó seems to be an important environmental factor in the life of *C. amazonicus*. My observations at the Jaú and Cuieiras rivers show that during low water, when large expanses of shoreline become exposed, *C. amazonicus* individuals concentrate on these margins where suitable microhabitats for foraging and basking become abundant. When the water begins to rise and several ponds are formed in the low igapó forests, it seems to gradually move into the forest, probably seeking stretches of margins of inland ponds where sunspots or clearings are available; during this period, foraging may occur in the shallows of these ponds. Only one *C. amazonicus* individual was found during high water, although during this period I failed to find the igapó margins where individuals could have been. If sunspots and clearings are scarce in the flooded igapó margins during high water, *C. amazonicus* may even decrease or interrupt activity until the water level goes down again. In an area south of Manaus, *C. amazonicus* individuals were observed sitting 10–15 m off ground on top of the ends of branches over the edges of a lake (L. J. Vitt, pers. comm.); thus, alternatively, this species may become more arboreal during high water. A radio-tracking study of *C. amazonicus* would certainly contribute significantly to our knowledge of its seasonal activity cycle. The biology of populations of the tropidurid *U. superciliosus* that inhabits areas of floodable forests also seems to be affected by the variation in water level; individuals seem to follow the margins of waterbodies as water level fluctuates (Howland *et al.*, 1990).

The swimming behaviour of *C. amazonicus* has been described as "strong, undulating movements of body and tail" (Hoogmoed & Lescure, 1975), or as "lateral movements of tail, while limbs are kept adpressed along body" (Ávila-Pires, 1995, quoting M. Martins). Additional observations of several swimming individuals corroborate the quotation in Ávila-Pires (1995), which was based on a single individual. The long (about twice as long as body length), laterally compressed tail of *C. amazonicus* (Fig. 1) may facilitate this serpentine swimming method, which resembles that of a caiman (suggestively, the local name of *C. amazonicus* in Brazilian Amazonia, jacarerana, means false caiman; see also Gasc, 1990). Vanzolini & Valencia (1965) suggested that the fringed toes of *C. amazonicus* could indicate that it walks on the water. However, I never observed this behaviour in *C. amazonicus*.

Besides the long, laterally compressed tail, other morphological traits in *C. amazonicus* seem to be associated with its swimming style: the relatively long neck (that makes it similar to varanids; Fig. 1; Goeldi, 1902), which could help in keeping a larger portion of the head outside the water while swimming; and the nostrils apparently closer to the tip of the snout than in other teiids (Goeldi, 1902). On the other hand, the evident dorsal crests and large convex scales found in *Dracaena*, which makes this species very similar to a caiman and seem to be adaptations for swimming, are not present in *C. amazonicus* (Fig. 1). An ecomorphological study of teiids would certainly reveal additional adaptations for aquatic habits in *Crocodilurus* and *Dracaena*.

It has been suggested that *C. amazonicus* may be non-heliothermic (Vanzolini, 1993), although this is not corroborated by the data presented herein. However, body temperature may drop significantly when basking lizards move into the water, although the water surface may reach relatively high temperatures (ca. 35°C) during the hottest hours of a sunny day in central Amazonia.

Crocodilurus amazonicus in the Jaú River feeds mostly on aquatic invertebrates (insects and crustaceans), but also on aquatic vertebrates (mainly fish), and shows wide food niches (8.1 and 7.5 for numerical and volumetric diet data, respectively). Wide food niches are widespread among teiids (Greene, 1982; Vitt, 1991; Vitt & Carvalho, 1992; Vitt & Colli, 1994) and *C. amazonicus* is no exception. For instance, the numerical and volumetric niche breadths of *C. amazonicus* were similar to or higher than those calculated by Vitt *et al.* (2000) for sympatric *A. ameiva* (8.9 and 4.1), *Kentropyx pelviceps* (2.4 and 2.2) and *K. altamazonica* (4.7 and 3.7) in western Amazonia (see similar results for *A. ameiva* and *K. altamazonica* in Vitt *et al.*, 2001, and Vitt & Colli, 1994). Despite their phylogenetic affinities, other aquatic or semiaquatic Amazonian lizards have relatively wide food niches: 15.2 and 8.6 (for numerical and volumetric data, respectively) in the gymnophthalmid *Neusticurus ecpleopus* (Vitt & Ávila-Pires, 1998) and 10.8 and 8.0 in the tropidurid *Uranoscodon superciliosus* (Howland *et al.*, 1990).

Although the food niche of *C. amazonicus* is relatively wide, both genera of primarily aquatic teiids, *Crocodilurus* and *Dracaena*, are specialized on aquatic prey to some degree. Although poorly studied, both species of *Dracaena* are considered mollusc specialists (Amaral, 1950; Vanzolini, 1961; Rand, 1964; Dixon & Soini, 1986). On the other hand, another teiid that inhabits aquatic habitats in Amazonia, *Kentropyx altamazonica* (rivers, igapós and várzeas; pers. obs.; Ávila-Pires, 1995; Vitt *et al.*, 2001), neither swims like *C. amazonicus* and *Dracaena* (instead, it runs over the water; Martins, 1996) nor feeds frequently on aquatic or

vertebrate prey (Vitt *et al.*, 2001; W. Y. Oda, pers. comm.).

Vertebrates form a considerable part of the diet in a few relatively unspecialized teiids (e.g. lizards in *Callopistes flavipunctatus*, Greene, 1982; frogs in *Kentropyx striatus,* Vitt & Carvalho, 1992, pers. obs.). Other teiids like *A. ameiva*, *Callopistes maculatus*, *Cnemidophorus lemniscatus*, *K. calcarata* and *Tupinambis* spp. may occasionally feed on small vertebrates (e.g. Greene, 1982; Vitt, 1991; Vitt & Carvalho, 1992; Vitt & Colli, 1994; Pianka & Vitt, 2003; M. Martins, pers. obs.). Vertebrates (fish, frogs and tadpoles) comprised about one third of the diet of *C. amazonicus.*

Piscivory is extremely rare in lizards (see review on lizard diets in Pianka & Vitt, 2003), being well known only in large aquatic monitors (e.g. Shine, 1986; Losos & Greene, 1988). *Crocodilurus amazonicus* is perhaps the only Neotropical lizard in which fish are important prey in the diet (23% of prey volume).

Feeding on aquatic prey occurs in some lizards that are associated with water bodies, but not in others. The tropidurid *U. superciliosus*, which is closely associated with river banks and forest streams, seems not to feed frequently on aquatic prey (Howland *et al.*, 1990; Gasnier *et al.*, 1994), although it may feed on prey floating on the surface film of rivers (Howland *et al.*, 1990). On the other hand, the gymnophthalmid *N. ecpleopus* feeds frequently on larvae of aquatic insects (Vitt *et al.*, 1998) and both species of *Dracaena* seem to feed heavily on aquatic molluscs (Amaral, 1950; Vanzolini, 1961; Rand, 1964; Dixon & Soini, 1986). The ingestion of fish, tadpoles, crabs, shrimps and several different species of aquatic insects indicates that *C. amazonicus* forages mainly in water. The ease with which *C. amazonicus* dives and the transparency of shallow water in igapó and várzea margins may facilitate underwater foraging. Furthermore, most of the prey eaten are nocturnal, indicating that prey may be disturbed by the lizard's movements by day. Alternatively, prey may be detected chemically, as teiids are characterized by a highly developed chemosensory system (see, e.g, Schwenk, 1995). In any case, how *C. amazonicus* manages to grasp fast moving prey like fish and shrimps remains to be studied.

Juvenile recruitment in *C. amazonicus* seems to occur early in the low water season in central Amazonia. If prey availability is higher during low water (when the igapó margins become exposed), recruitment at the onset of this period would guarantee a good food supply for juveniles. At least in captivity, growing is rapid in *C. amazonicus* (fed with meat): three juveniles with SVL ca. 80–100 mm, collected in November 1995, at Jaú River, attained ca. 180–230 mm in about two years (October 1997; A. S. Abe, pers. comm.).

Although the present study provides new information on the ecology of *C. amazonicus*, it was conducted in a very small portion of its geographical distribution. Thus, further studies in other areas, especially in várzea regions, would certainly provide important new information on this interesting species. The occurrence of *C. amazonicus* in many preserved areas in Brazil (the entire Jaú river basin, for instance, lies within one of the largest Brazilian parks, with over two million hectares) and adjacent countries may facilitate its preservation in the future, when inevitably most unprotected areas in Amazonia will be clear cut or at least highly disturbed.

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REFERENCES

Amaral, A. (1950). Two new South American lizards. *Copeia* **1950**, 281-284.

Ávila-Pires, T. C. S. (1995). Lizards of Brazilian Amazonia (Reptilia: Squamata). *Zoologische Verhandelingen (Leiden)* **299**, 1-706.

Bartlett, R. D. & Bartlett, P. (2003). *Reptiles and Amphibians of the Amazon: An Ecotourist's Guide.* Gainesville: University Press of Florida.

Crump, M. L. (1971). Quantitative analysis of the ecological distribution of a tropical herpetofauna. *Occasional Papers of the Museum of Natural History University of Kansas* **3**, 1-62.

Dixon, J. R. & Soini, P. (1986). *The Reptiles of the Upper Amazon Basin, Iquitos Region, Peru.* Milwaukee: Milwaukee Public Museum.

Gasc, J.-P. (1990). *Les Lézards de Guyane*. Editions Raymond Chabaud, Paris.

Gasnier, T. R., Magnusson, W. E. & Lima, A. P. (1994). Foraging activity and diet of four sympatric lizard species in a tropical rainforest. *Journal of Herpetology* **28**, 187-192.

Goeldi, E. (1902). Lagartos do Brasil. *Boletim do Museu Paraense Emílio Goeldi* **3**, 499-560.

Greene, H. W. (1982). Dietary and phenotypic diversity in lizards: why are some organisms specialized? In *Environmental Adaptation and Evolution: A Theoretical and Empirical Approach*, 107-128. Mossakowski, D. and Roth, G. (Eds). Stuttgart: Gustav Fischer-Verlag.

Hoogmoed, M. S. & Lescure, J. (1975). An annotated checklist of the lizards of French Guiana, mainly based on two recent collections. *Zoologische Mededelingen (Leiden)* **49**, 141-171.

Howland, J. M., Vitt, L. J. & Lopez, P. T. (1990). Life on the edge: the ecology and life history of the tropidurine

iguanid lizard *Uranoscodon superciliosum*. *Canadian Journal of Zoology* **68**, 1366-1373.

Losos, J. B. & Greene, H. W. (1988). Ecological and evolutionary implications of diet in monitor lizards. *Biological Journal of the Linnean Society* **35**, 379-407.

Martins, M. (1996). Defensive tactics in lizards and snakes: the potential contribution of the Neotropical fauna. *In Anais do XIV Encontro Anual de Etologia,* 185-199. Del-Claro, K. (Ed.). Uberlândia: Sociedade Brasileira de Etologia.

Martins, M. & Oliveira, M. E. (1998). Natural history of snakes in forests of the Manaus region, central Amazonia, Brazil. *Herpetological Natural History* **6**, 78-150.

Pianka, E. R. & Vitt, L. J. (2003). *Lizards: Windows to the Evolution of Diversity*. Berkeley: University of California Press.

Rand, A. S. (1964). An observation on *Dracaena guianensis* foraging underwater. *Herpetologica* **20**, 207.

Sartorius, S. S., Vitt, L. J. & Colli, G. R. (1999). Use of naturally and anthropogenically disturbed habitats in Amazonian rainforest by the teiid lizard *Ameiva ameiva*. *Biological Conservation* **90**, 91-101.

Schwenk, K. (1995). Of tongues and noses: chemoreception in lizards and snakes. *Trends in Ecology and Evolution* **10**, 7-12.

Shine, R. (1986). Food habits, habitats and reproductive biology of four sympatric species of varanid lizards in tropical Australia. *Herpetologica* **42**, 346-360.

Vanzolini, P. E. (1961). Notas bionômicas sobre *Dracaena guianensis* no Pará (Sauria, Teiidae). *Papéis Avulsos de Zoologia (São Paulo)* **14,** 237-241.

Vanzolini, P. E. (1972). Miscellaneous notes on the ecology of some Brasilian lizards (Sauria). *Papéis Avulsos de Zoologia (São Paulo)* **26**, 83-115.

Vanzolini, P. E. (1993). Mass–length relationships in macroteiid and other lizards are insensitive to ecology and geography (Sauria, Teiidae). *Papéis Avulsos de Zoologia (São Paulo)* **38,** 77-89.

Vanzolini, P. E. & Valencia, J. (1965). The genus *Dracaena*, with a brief consideration of macroteiid relationships (Sauria, Teiidae). *Arquivos de Zoologia (São Paulo)* **13**, 7-35.

Vitt, L. J. (1991). Ecology and life history of the widely foraging lizard *Kentropyx calcarata* (Teiidae) in Amazonian Brazil. *Canadian Journal of Zoology* **69**, 2791-2799.

Vitt, L. J. & Ávila-Pires, T. C. S. (1998). Ecology of two sympatric species of *Neusticurus* (Sauria: Gymnophthalmidae) in the western Amazon of Brazil. *Copeia* **1998**, 570-582.

Vitt, L. J. & Carvalho, C. M. (1992). Life in the trees: the ecology and life history of *Kentropyx striatus* (Teiidae) in the lavrado area of Roraima, Brazil, with comments on tropical teiid life histories. *Canadian Journal of Zoology* **70**, 1995-2006.

Vitt, L. J. & Colli, G. R. (1994). Geographical ecology of a neotropical lizard: *Ameiva ameiva* (Teiidae) in Brazil. *Canadian Journal of Zoology* **72**, 1986-2008.

Vitt, L. J., Sartorius, S. S., Ávila-Pires, T. C. S., Espósito, M. C. & Miles, D. B. (2000). Niche segregation among sympatric Amazonian teiid lizards. *Oecologia* **122**, 410-420.

Vitt, L. J., Sartorius, S. S., Ávila-Pires, T. C. S. & Espósito, M. C. (2001). Life at the river's edge: ecology of *Kentropyx altamazonica* in Brazilian Amazonia. *Canadian Journal of Zoology* **79**, 1855-1865.

Vitt, L. J., Zani, P. A., Caldwell, J. P., Araújo, M. C. & Magnusson, W. E. (1997). Ecology of whiptail lizards (*Cnemidophorus*) in the Amazon region of Brazil. *Copeia* **1997**, 745-757.

Vitt, L. J., Zani, P. A., Caldwell, J. P. & Carrillo, E. O. (1995). Ecology of the lizard *Kentropyx pelviceps* (Sauria: Teiidae) in lowland rain forest of Ecuador. *Canadian Journal of Zoology* **73**, 691-703.

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REPRODUCTIVE ECOLOGY OF A MEDITERRANEAN RATSNAKE, THE LADDER SNAKE *RHINECHIS SCALARIS* (SCHINZ, 1822)

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Organisms that produce more than one newborn at every reproductive event must choose between two options with respect to their reproductive output: to produce a few large or many small young. The decision will be influenced by the spectrum of prey sizes available to young. The ladder snake (*Rhinechis scalaris*), a heavy-bodied Mediterranean colubrid, is well suited for the study of its reproductive ecology under this cue: the species consumes only endothermic prey and, thereafter, hatchlings of this gape-size-limited predator must be large enough to prey on small mammals. We analysed the reproductive ecology of this species, a quasi-endemic to the Iberian Peninsula, by studying a large sample of specimens collected in the south-eastern Iberian Peninsula. Most adult females (83.3%) reproduced in sequential years, with vitellogenesis beginning in early spring, and oviposition occurring in the first half of July. In contrast to the general rule for most temperate snakes, no depletion in fat bodies was observed during the period of vitellogenesis, females exhibiting a very high level of fat-body reserves throughout all months of the activity period. When compared with other Mediterranean species, hatching occurred very late in the activity season (October), newborns were rather heavy bodied, with very high fat-body levels, and apparently did not feed until the following spring. From our dataset, we suggest that female *R. scalaris* produce hatchlings large enough to enter hibernation without feeding, perhaps increasing in this way the survival rate of juveniles in their first calendar year. They probably need to devote their reserves at hatching to growth and to better face their first, bulky prey.

Key words: colubrid, fat bodies, hatching time, hatchling size

INTRODUCTION

Snakes have begun to attract increasing attention from many researchers as model organisms in ecological studies (Shine & Bonnet, 2000). Reproductive ecology is among the topics most addressed by researchers, as snakes exhibit wide diversity in reproductive strategies, in terms of frequency of reproduction, strategies to fuel reproductive energy cost ("capital" versus "income" breeders, as proposed by Drent & Daans, 1980), and clutch size or hatchling size (Ford & Seigel, 1989). Moreover, noteworthy links can be found between the reproductive ecology of a snake and other traits of its natural history, such as feeding ecology (Shine, 2003). Certainly, food intake determines the reproductive output of snakes (Ford & Seigel, 1989).

The reproductive output of organisms, in the simplest analysis, can be split into two possibilities: to produce a few large young, or to produce many small young (Roff, 1992; Gregory & Larsen, 1993). The choice would be mediated by the spectrum of prey sizes available to the hatchlings (Nussbaum, 1981). With a given amount of energy supplied for reproductive output, a species that preys on a wide range of prey types and size would choose between the two possibilities. However, species with a narrower spectrum in prey type and/or size, particularly if available prey for neonates are large, would find the choice of producing a few large hatchlings to be more adaptive (Sun *et al.*, 2002). The ladder snake, *Rhinechis scalaris*, is a rather large, heavy-bodied and oviparous colubrid, that inhabits the Iberian Peninsula, south-eastern France, and the westernmost part of Italy (Pleguezuelos & Cheylan, 1997), and is a suitable organism for analysing links between reproductive and feeding ecology. This colubrid has the peculiarity of feeding only on endotherms, mostly small mammals (Cheylan & Guillaume, 1993). As opposed to other medium-sized Mediterranean predators, whether snakes (Díaz-Paniagua, 1976; Saint Girons, 1980) or other animals (Jacksic *et al.*, 1982), *R. scalaris* does not undergo a typical ontogenetic shift in dietary habits. While young individuals of many snake species prey on small-sized, slender-bodied ectotherms, and large individuals prey on bulky prey such as small mammals, both young and adult *R. scalaris* prey almost exclusively on small mammals (Pleguezuelos, 1998). Thus, we would expect *R. scalaris,* a gape-limited predator, to produce few large hatchlings to minimize predator/prey size constraints in the first period of individual development (King, 1993).

Here we study a large sample of free-ranging animals and museum specimens from the south-eastern Iberian Peninsula to determine the reproductive ecology of this snake; individuals were also checked for stomach contents to obtain an index of the feeding rate of the species. We address the following main questions: (1) Do male and female reproduce in sequential years? (2) Is there fat-body cycling in sexually mature individuals, and if so, is cycling tied to vitellogenesis in females? (3) Is clutch size related to maternal size? Does the female produce large clutches of small

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hatchlings, or small clutches with large hatchlings? 4) Do newborns feed in their first calendar year, or rely on the vitellogenic reserves at the beginning, delaying feeding until next calendar year?

This species is remarkably differentiated from other ratsnakes in morphology, plasma protein and mitochondrial-DNA sequence (Helfenberger, 2001; Lenk *et al.*, 2001; Utiger *et al.*, 2002). In this sense, there is evidence for a separate position of this taxon within the genus *Elaphe*, derived in the revalidation of the monotypic genus *Rhinechis* Michaeles, 1833 (Helfenberger, 2001; Utiger *et al.*, 2002). For this reason, we adopt the new taxonomical proposal and use the combination *Rhinechis scalaris* for the species.

MATERIAL AND METHODS

The field study was conducted in the Granada Depression and closely surrounding areas, a region of approx. 3000 km^2 in the south-eastern Iberian Peninsula (36° 55'–37° 20' N, 3° 30'–4° 15' W), which spans altitudes between 450 and 1200 m asl. Climate is typically Mediterranean; mean minimum temperature ranged from 1.9 to 4.4 °C in winter (January), the mean maximum temperature from 31.0 to 35.6 °C in summer (July). The mean annual temperature ranged from 12.5 to 14.3 °C, and average yearly rainfall ranged from 355.4 to 448.0 mm (data from the Cartuja weather station [37° 12' N, 3° 36' W], representative of the study area).

Field sampling was conducted from 1993 to 2000, within the framework of a larger study on the snake fauna of the region (details in Feriche, 1998). We made searches 3–4 field days per month (c. 6 hr each), throughout all months of the year. Specimens were collected from among those killed by local people and by traffic. A total of 320 specimens were collected in this way, with vouchers preserved in alcohol and deposited in the University of Granada (DBAG). Although the species is hard to find and difficult to catch, we hand-captured specimens alive when possible (25 individuals), although they provided little information on reproduction. We also analysed some specimens from the collections of the Estación Biológica de Doñana, Seville (EBD; n=20), and Museo Nacional de Ciencias Naturales, Madrid (MNCN; n=3), collected in the study area. In total, we examined 368 specimens (202 males, 166 females). Because this study spanned nine years, we assumed that the reproductive cycle of the species remained stable in the study area over the years.

In all specimens, snout-vent length (SVL) and tail-length (TL) were measured with a cord (± 1 mm). Live snakes were sexed by the SVL/TL relationship and the shape of the base of the tail (Feriche *et al.*, 1993), and females were gently palpated in the rear abdomen to check for pregnancy. All specimens from collections were dissected, and we took the following measurements: longest, medium and shortest axes of the right testis (± 0.1 mm) in males; diameter of the largest follicle or oviductal egg (± 0.1 mm) in females; and fat-body size in both sexes. In males, testicular volume (TV) was estimated using the formula for the volume of a flattened ellipsoid (Mayhew, 1963). In that sex, size at maturity and the spermatogenic cycle were determined by relating TV with spermatogenic activity (Seigel & Ford, 1987). To calculate size at maturity and reproductive cycle in males, we used the right testis, always larger and in front of the left one (Feriche, 1998). Because TV increases significantly with SVL, TV was standardized to avoid bias by the residuals of the regression of TV (log transformed) on SVL. To calculate size at maturity in females, we observed the shift in follicle size with respect to body length (SVL) in the whole sample of females. Because we were unable to weigh fat bodies accurately from some road-killed specimens, or to remove fat bodies from museum specimens, we scored the fat-body level in five visual categories: 0: no traces of fat; 1: small traces of fat among the intestine loops; 2: fat bodies covering less than half of the intestinal surface; 3: fat bodies covering more than half of the intestinal surface; and 4: a continuous fat layer in the ventral zone of the abdominal cavity (Pleguezuelos & Feriche, 1999). We also checked for stomach content to obtain an index of feeding frequency, and correlate it to some traits of the reproductive ecology of the species: in live snakes by gentle palpation of the fore abdomen to force regurgitation in the case of recently ingested food, and in voucher specimens by an incision in the stomach. Measurements were taken only from well-preserved specimens and/or organs, resulting in different sample sizes for various measurements. Mean values are followed by ± 1 SD.

RESULTS

The SVL of *R. scalaris* in the study area ranged from 233 to 1385 mm (mean=719.3±206.9 mm, n=368), and weight from 11.2 to 1720.2 g (mean=239.9±233.0 g, n=288). Testicular volume was lower than average in specimens under 400 mm SVL, while males larger than 500 mm SVL had higher than average TV, indicating a testicular recrudescence (Fig. 1A). Thus, we classified males > 450 mm SVL as sexually mature. The smallest female that showed signs of reproductive activity (follicles >13 mm in length) was 660 mm SVL (Fig. 2A), and this was tentatively taken as the size at which females attained sexual maturity.

With respect to the reproductive cycle, in adult males testicular recrudescence began in mid-May, peaked in July and August, and decreased through autumn, testes volume reaching its smallest size during winter (Fig. 1B). The beginning of the mating period was deduced from the observation of a mating ball in the wild, which occurred at the end of March. The annual TV and the mating period of this species indicates that males have a seasonal and aestival spermatogenic cycle (*sensu* Saint Girons, 1982). From Fig. 1B, we also deduced that most adult males underwent spermatogenesis in sequential years. In females, the reproductive cycle was also sea-

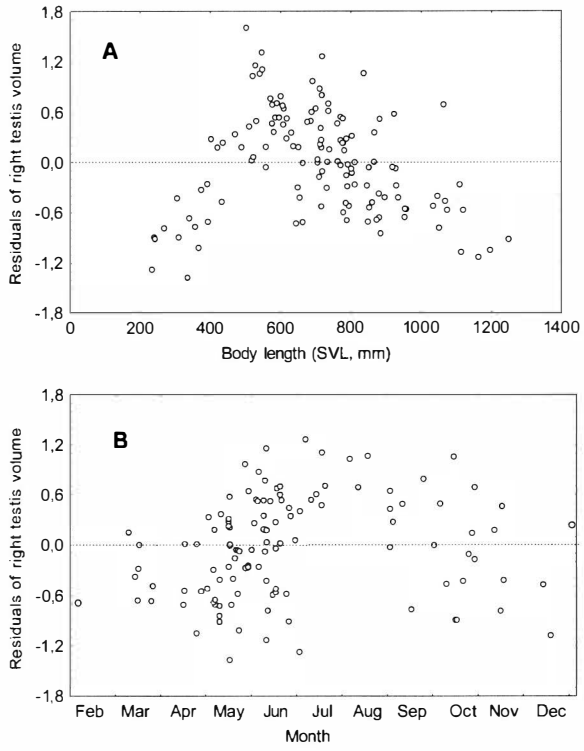

FIG. 1. Testicular recrudescence in males of the ladder snake *(Rhinechis scalaris)* in the Depression of Granada. Residual scores of the right testis volume vs. (A) body size (snout-vent length [SVL]; all individuals considered; n=170), and (B) month of the year (only reproductive individuals, SVL>450 mm; n=103). Each data point represents one individual.

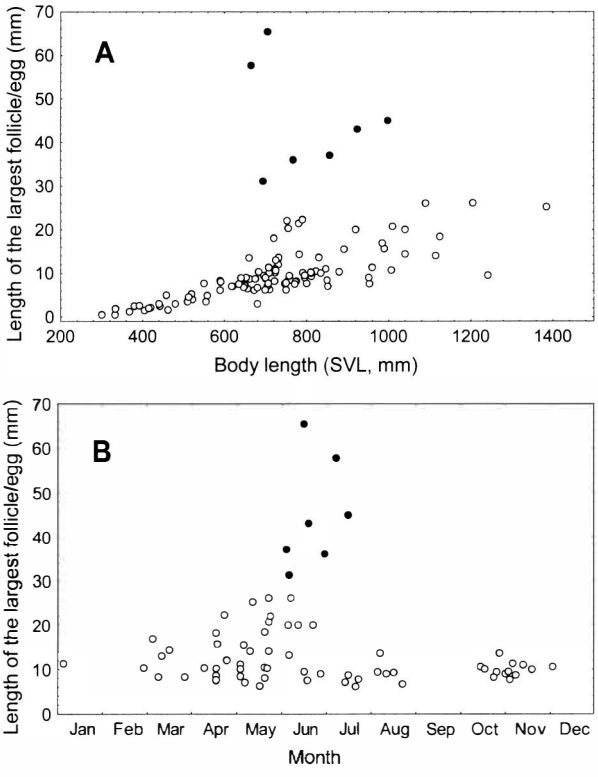

FIG. 2. Length of the largest follicle (open circle) and oviductal egg (solid circle) in females of the ladder snake (*Rhinechis scalaris*), in the Depression of Granada, plotted against (A) body size (snout-vent length [SVL]; all individuals considered; n=131), and (B) month of the year (only reproductive individuals, SVL > 660 mm; n=68).

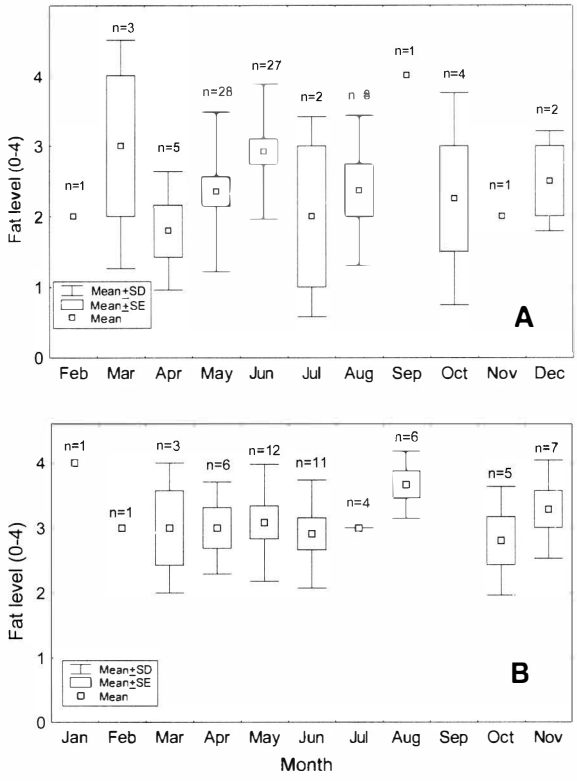

FIG. 3. Abdominal fat level for reproductive males (A) and females (B) of the ladder snake (*Rhinechis scalaris*) in the Depression of Granada. Fat-body level is scored in five categories, from 0 to 4 (see Methods section for more details).

sonal. Vitellogenesis begins in April, and oviductal eggs were present during June and the first half of July (Fig. 2B). Neither of the adult females from the second half of July and August contained oviductal eggs (Fig. 2B). Therefore, we estimated oviposition in the field to occur in the first half of July.

In mature males and females, abdominal fat bodies were rather large and homogeneous over the year (K-W test; males, $H_{6,81}$=2.22, P=0.3; females, $H_{7,55}$=5.49, P=0,6; in both analyses, winter months pooled because of small sample size; Fig. 3). Mature females had larger fat bodies than mature males (M-W U test, U=1922.5, P<0.003) or immature females (U=429.5, P<0.0001). In mature females, frequency of specimens with prey in the stomach did not differ when those carrying enlarged follicles were compared with those outside the vitellogenesis period (2 x 2 table, χ^2=0.10, n=98, P=0.9). Thus, females apparently continued to feed during the vitellogenesis period.

Clutch size ranged from 4 to 14 eggs (mean=7.4 ± 2.8, n=19), with a significant positive relationship between body length and clutch size (r=0.690, n=19, P<0.001; clutch size = 0.0099 × SVL−1.605). Of the 27 sexually mature females in the reproductive period, 20 (74%) contained large vitellogenic follicles or oviductal eggs. The percentage of annual reproductive females became higher (83.3%) when discounting three of the seven non-reproductive females, with body size close to the minimum size to be sexually mature, this size (660 mm SVL) being an approximation more than a thresh-

old value. Hence, most of the sexually mature females reproduced in sequential years.

In the study area, the first hatchlings were recorded in the field on 5 October after intensive field searching. Taking into account the oviposition date and that newborns remain in the nest for 7–13 days, until first ecdysis (Blázquez, 1994), we deduced the incubation period to be approximately 65 days. Offspring size, calculated from hatchlings found in the field in their first calendar year, ranged from 240 to 310 mm SVL (mean=277.0±28.3, n=11), and offspring weight ranged from 11.2 to 17.5 g (mean=15.1±2.2, n=6). The fat-body level of hatchlings was rather high (mean 3.4±0.84, range 2–4, n=10). None of the 11 newborns found in the field had gut content. Thus, we suggest that newborns enter their first hibernation period relying only on their vitellogenic reserves. In the spring of their second calendar year, young exhibited a fat-body level (mean=2.0±1.1, range 0–4, n=12) lower than in the hatchling period (M-W U test, Z=1.94, P=0.052). Although the comparison did not reach significance, it suggests depletion in fat-body level during the first hibernation period.

DISCUSSION

As observed in other large oviparous colubrids (Parker & Plummer, 1987), or in other species of the former genus *Elaphe* (Fitch, 1963), male *R. scalaris* matured at shorter absolute and relative SVL (SVL at maturity as a percentage of maximum size) than did females. In snakes with no sexual dimorphism in body size, the different size at maturity has been interpreted as the result of females requiring a high threshold level of energy stores to initiate reproduction (Duvall *et al.*, 1992). The finding in the present study that mature females exhibited higher fat-body levels than immature specimens of both sexes and adult males supports this interpretation.

Males exhibited an aestival cycle, in which sperm overwinters in the ducts deferent until the mating period, in spring of the next year. The aestival cycle is typical of snakes from the Temperate Zone (Seigel & Ford, 1987), and fits well with the northern Mediterranean distribution of *R. scalaris*. The aestival spermatogenic cycle allows this species to mate early the next year, immediately after emerging from winter dormancy, as confirmed by our observation of mating activity.

Females exhibit a seasonal reproductive cycle, as is general in snakes from the temperate zones (Saint Girons, 1982). This highly seasonal pattern observed in the reproduction of many snakes should be guided by seasonal variation in available resource levels, hatchling survival rates (Shine, 2003), or thermal requirements during embryogenesis (Saint Girons, 1982). In *R. scalaris*, vitellogenesis occurs in late spring, when resource availability peaks in terrestrial Mediterranean habitats, with oviposition in early summer, and embryogenesis in the warmer months of the year (Feriche, 1998). Since no specimen was found with gut content in their first calendar year (autumn season), reproduction in this species is most likely not timed to favour the access of newborns to feeding resources.

In reptiles, ectothermic vertebrates with relatively low metabolisms, the accumulation of body reserves to fuel reproductive processes is a common phenomenon (Bonnet *et al.*, 1998*b*). Thus, a major issue in studying snakes that inhabit temperate regions, with a seasonal reproductive cycle, is to understand the seasonal variation in energy related to reproduction (Congdon, 1989; Santos & Llorente, 2004). In most temperate snakes fat bodies are large in spring, reach a low point in late spring to early summer, and then increase gradually until hibernation (Seigel & Ford, 1987; Pleguezuelos & Feriche, 1999; Santos & Llorente, 2001). However, surprisingly, fat-body cycling in female *R. scalaris* was not tied to the timing of the reproductive cycle. In this species, stored lipids must contribute to the energy needed for follicular maturation and egg yolking, but not to the degree of depleting fat-body levels during the reproductive period. This pattern mirrors that observed in some tropical snakes (Seigel & Ford, 1987), for which it has been suggested that foraging success is high enough to preclude the necessity of resorting to fat reserves for reproduction (Berry & Lim, 1967). Though *R. scalaris* is not tropical, the cue for the lack of fat-body cycling in tropical snakes (high foraging success) would apply to this species (see also Valverde, 1967; authors' unpublished data). Mature *R. scalaris* females during vitellogenesis continue feeding (but not in the late pregnancy stage; M. C. Blázquez, pers. comm.), an uncommon phenomenon in snakes (Agrimi & Luiselli, 1994; Gregory *et al.*, 1999; Rohr, 2003). Thus, the high fat-body level of mature females at the beginning of vitellogenesis suggests that the species functions as a capital breeder for fuelling the breeding process, as occurs in most ectotherms (Bonnet *et al.*, 1998*a*). Taking into account that females continue to feed during vitellogenesis, we should consider that the species is an income breeder to some degree. However, this major issue in the reproductive ecology of ectothermic vertebrates is in reality far from a simple dichotomy (capital vs. income). Many species must rely on intermediate possibilities, or simply be "facultative income" breeders, as has been recently found for some colubrids and viperids (Lourdais *et al.*, 2002; Reading, 2004; Santos & Llorente, 2004); *R. scalaris* would be another example.

The ladder snake exhibited a low relative fecundity (mean clutch size vs. female body length), as is normal in terrestrial colubrids when compared with aquatic ones (Seigel & Ford, 1987). At least the mean and range for the clutch size found here for this species were within the values recorded for other Palaearctic species of the former genus *Elaphe* (Schulz, 1996; Rugiero, 1998; Rugiero *et al.*, 1998). However, clutch size is not the sole measure of snake reproductive investment (Seigel *et al.*, 1986). In *R. scalaris*, egg size is rather

large (Cheylan & Guillaume, 1993; see also Fig. 2B), and relative clutch mass is considerable, representing between 31 and 47% of female body mass (Blázquez, 1994; Cheylan & Guillaume, 1993; data for comparison in Seigel *et al.*, 1986). A reproductive strategy in female snakes that invest substantial energy in reproduction is to lower the frequency of reproduction (Shine, 2003). Nevertheless, our results, supported by a large sample size, indicate that most mature, good-sized females reproduce in sequential years, as is general in active searcher snakes (such as the species analysed here; Pleguezuelos, 1998), compared to ambush foragers (Webb *et al.*, 2003).

Besides reproducing in sequential years, females of *R. scalaris* lay large eggs that give birth to large, heavy-bodied hatchlings (average hatchling body weight as a percentage of average adult body weight scored 5.1% in *R. scalaris*, and 2.5--4.1% in other western Mediterranean medium-sized colubrids; data from Feriche, 1998 and Fahd, 2001).

In temperate snakes, late-born hatchlings may have fewer opportunities to feed before winter, but normally they benefit from larger energy reserves (Sun *et al.*, 2002). According to our data, *R. scalaris* agrees with this strategy, which combines hatching date, newborn fat reserves and feeding rate. Hatchlings of *R. scalaris* born later in the year than hatchlings or offspring of any other western Mediterranean snake (see reviews in Böhme, 1993, 1999) exhibit very high fat-body levels, and apparently do not feed in their first calendar year (present study). The large amount of vitellogenic reserves attached to the gut in hatchlings must fuel growth until spring, in the next calendar year. This has been defined as extended parental care in the form of yolk reserves that remain in the hatchling after it leaves the egg (Congdon, 1989). In any case, this conclusion should be regarded with caution, because of the small sample size for newborns, and more information is needed on hatchling survival and growth in order to draw definitive conclusions concerning the adaptive significance of delayed hatching dates in snakes.

Although the interrelationships among clutch size, hatchling size, maternal body size and maternal body condition are complex and should be addressed by a multivariate analysis (King, 1993; Sun *et al.*, 2002), we can draw certain conclusions from our dataset on *R. scalaris*: (1) most mature, large-sized females reproduce in sequential years; (2) reproductive output seems to be focused on a few but large-sized and fatty hatchlings; (3) these hatchlings appear to enter their first hibernation period without feeding, probably enhancing in this way their survivorship. Meanwhile, they devote time and reserves to growth, in order to face their first bulky prey (small mammals).

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REFERENCES

Agrimi, U. & Luiselli, L. (1994). Ecology of the snake *Coronella girondica* (Reptilia: Colubridae) in Central Italy. *Vie et Milieu* **44**, 203-210.

Berry, P. Y. & Lim, G. S. (1967). The breeding pattern of the puff-faced water snake *Homalopsis buccata* Boulenger. *Copeia* **1967**, 307-313.

Blázquez, M. C. (1994). Notes on the reproduction of *Elaphe scalaris* (Schinz, 1822) in Southern Spain. *Herpetological Journal* **4**, 109-111.

Böhme, W. (Ed.) (1993). *Handbuch der Reptilien und Amphibien Europas. Schlangen (Serpentes), I.* Wiesbaden: Aula-Verlag.

Böhme, W. (Ed.) (1999). *Handbuch der Reptilien und Amphibien Europas. Schlangen (Serpentes), II.* Wiesbaden: Aula-Verlag.

Bonnet, X., Bradshaw, S. D. & Shine, R. (1998a). Capital versus income breeding: an ectothermic perspective. *Oikos* **83**, 333-341.

Bonnet, X., Shine, R., Naulleau, G. & Vacher-Vallas, M. (1998b). Sexual dimorphism in snakes: different reproductive roles favour different body plans. *Proceedings of the Royal Society of London B* **265**, 179-183.

Cheylan, M. & Guillaume, C. P. (1993). *Elaphe scalaris* (Schinz, 1822) – Treppennatter. In: *Handbuch der Reptilien und Amphibien Europas. Band 3/I: Schlangen (Serpentes) I (Typhlopidae, Boidae, Colubridae 1: Colubrinae)*, 397-429. Böhme, W. (Ed). Wiesbaden: Aula-Verlag.

Congdon, J. D. (1989). Proximate and evolutionary constraints on energy relations of reptiles. *Physiological Zoology* **62**, 356-373.

Díaz-Paniagua, C. (1976). Alimentación de la culebra bastarda (*Malpolon monspessulanus*, Ophidia, Colubridae) en el S.O. de España. *Doñana, Acta Vertebrata* **3**, 113-127.

Drent, R. H. & Daans, S. (1980). The prudent parent: energetic adjustment in avian breeding. *Ardea* **68**, 225-252.

Duvall, D., Arnold, S. J. & Schuett G. W. (1992). Pitviper mating systems: ecological potential, sexual selection and microevolution. In: *Biology of the Pitvipers*, 321-336. Campbell, J. A. & Brodie, E. D. (Eds). Tyler, Texas: Selva.

Fahd, S. (2001). *Biogéographie, morphologie et écologie des ophidiens du Rif (Nord du Maroc).* PhD thesis. Tetouan: University of Abdelmalek Essaâdi.

Feriche, M. (1998). *Ecología de la Reproducción en Colúbridos del Sureste de la Península Ibérica.* PhD thesis. Granada: University of Granada.

Feriche, M., Pleguezuelos, J. M. & Cerro, A. (1993). Sexual dimorphism and sexing of Mediterranean colubrids based on external characteristics. *Journal of Herpetology* **27**, 357-362.

Fitch, H. S. (1963). Natural history of the black rat snake (*Elaphe o. obsoleta*) in Kansas. *Copeia* **1963,** 649-658.

Ford, N. B. & Seigel, R. A. (1989). Phenotypic plasticity in reproductive traits: evidence from a viviparous snake. *Ecology* **70**,1768-1774.

Gregory, P. T., Crampton, L. H. & Skebo, K. M. (1999). Conflicts and interactions among reproduction, thermoregulation and feeding in viviparous reptiles: are gravid snakes anorexic? *Journal of Zoology* **248**, 231-241.

Gregory, P. T. & Larsen, K. W. (1993). Geographic variation in reproductive characteristics among Canadian populations of the common garter snake (*Tamnophis sirtalis*). *Copeia* **1993**, 946-958.

Helfenberger, N. (2001). Phylognetic relationships of Old World ratsnakes based on visceral organ topography, osteology, and allozyme variation. *Russian Journal of Herpetology* **8 (suppl.)**, 1-64.

Jacksic, F. M., Greene, H. W., Schwenk, K. & Seib, R. L. (1982). Predation upon reptiles in Mediterranean habitats of Chile, Spain and California: a comparative analysis. *Oecologia* **53**, 152-159.

King, R. B. (1993). Determinants of offspring number and size in the brown snake, *Storeria dekayi*. *Journal of Herpetology* **27**, 175-185.

Lenk, P., Joger, U. & Wink, M. (2001). Phylogenetic relationships among European ratsnakes of the genus *Elaphe* Fitzinger based on mitochondrial DNA. *Amphibia-Reptilia* **22**, 329-339.

Lourdais, O., Bonnet, X., Shine, R., Denardo, D., Naulleau, G. & Guillon, M. (2002). Capital-breeding and reproductive effort in a variable environment: a longitudinal study of a viviparous snake. *Journal of Animal Ecology* **71**, 470-479.

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

Nussbaum, R. A. (1981). Seasonal shift in clutch size and egg size in the side-blotched lizard, *Uta stansburiana* (Baird and Girard). *Oecologia* **49,** 8-13.

Parker, W. S. & Plummer, M. V. (1987). Population ecology. In: *Snakes, Ecology and Evolutionary Biology*, 253-301. Seigel, R. A., Collins, J. T. & Novak, S. S. (Eds). New York: Macmillan.

Pleguezuelos, J. M. (1998). *Elaphe scalaris* (Schinz, 1822). In: *Fauna Ibérica, vol. 10*, 390-407. Ramos, M.A. *et al.* (Eds). Madrid: Museo Nacional de Ciencias Naturales. C.S.I.C.

Pleguezuelos, J. M. & Cheylan, M. (1997). *Elaphe scalaris* (Schinz, 1822). In: *Atlas of Amphibians and Reptiles in Europe*, 360-361. Gasc, J. P. (Ed). Paris: Societas Europaea Herpetologica & Muséum National d'Histoire Naturelle.

Pleguezuelos, J. M. & Feriche, M. (1999). Reproductive ecology of the horseshoe whip snake, *Coluber hippocrepis*, in the southeast of the Iberian Peninsula. *Journal of Herpetology* **33**, 202-207.

Reading, C. J. (2004). The influence of body condition and prey availability on female breeding success in the smooth snake, *Coronella austriaca* Laurenti. *Journal of Zoology* **264**, 61-67.

Roff, D. A. (1992). *The Evolution of Life Histories*. New York: Chapman & Hall.

Rohr, D. H. (2003). Reproductive trade-offs in the elapid snakes *Austrelaps superbus* and *Austrelaps ramsayi*. *Canadian Journal of Zoology* **79**, 1030-1037.

Rugiero, L. (1998). Food habits and reproduction of the snake *Elaphe quatuorlineata* in southern Italy. *Amphibia-Reptilia* **19**, 439-442.

Rugiero, L., Capizzi, D. & Luiselli, L. (1998). Aspects of the ecology of the leopard snake *Elaphe situla* in southeastern Italy. *Journal of Herpetology* **32**, 626-630.

Saint Girons, H. (1980). Selective modifications in the diet of vipers (Reptilia: Viperidae) during growth. *Amphibia-Reptilia* **1**, 127-136.

Saint Girons, H. (1982). Reproductive cycles of male snakes and their relationships with climate and female reproductive cycles. *Herpetologica* **38**, 5-16.

Santos, X. & Llorente, G. A. (2001). Seasonal variation in reproductive traits of the oviparous water snake, *Natrix maura,* in the Ebro Delta of northeastern Spain. *Journal of Herpetology* **35**, 653-660.

Santos, X. & Llorente, G. A. (2004). Lipid dynamics in the viperine snake, *Natrix maura*, from the Ebro Delta (NE Spain). *Oikos* **105**, 132-140.

Schulz, K. D. (1996). *A Monograph of the Colubrid Snakes of the Genus* Elaphe *Fitzinger*. Königstein: Koeltz.

Seigel, R. A., Fitch, H. S. & Ford, N. B. (1986). Variation in relative clutch mass in snakes among and within species. *Herpetologica* **42**, 179-185.

Seigel, R. A. & Ford, N. B. (1987). Reproductive ecology. In: *Snakes, Ecology and Evolutionary Biology*, 210-252. Seigel, R. A., Collins, J. T. & Novak S. S. (Eds). New York: Macmillan.

Shine, R. (2003). Reproductive strategies in snakes. *Proccedings of the Royal Society of London B* **270**, 995-1004.

Shine, R. & Bonnet, X. (2000). Snakes: a new "model organism" in ecological research? *Trends in Ecology and Evolution* **15**, 221-222.

Sun, L.-X., Shine, R., Debi, Z. & Zhengren, T. (2002). Low cost, high output: reproduction in an insular pit-viper (*Gloydius shedaoensis*, Viperidae) from north-eastern China. *Journal of Zoology* **256**, 511-521.

Utiger, U., Helfenberger, N., Schätti, B., Schmidt, C., Ruf, M. & Ziswiler, V. (2002). Molecular systematics and phylogeny of Old and New World ratsnakes, *Elaphe* auct., and related genera (Reptilia, Squamata, Colubridae). *Russian Journal of Herpetology* **9**,105-124.

Valverde, J. A. (1967). Estructura de una comunidad de vertebrados terrestres. *Monografías de la Estación Biológica de Doñana* **1**, 1-218.

Webb, J. K., Brook, B. & Shine, R. (2003). Does foraging mode influence life history traits? A comparative study of growth, maturation and survival of two species of sympatric snakes from southeastern Australia. *Austral Ecology* **28**, 601-610.

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A NEW SPECIES OF ARBOREAL *LEPTOPELIS* (ANURA: ARTHROLEPTIDAE) FROM THE FORESTS OF WESTERN KENYA

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A new species of arboreal *Leptopelis* is described from Kakamega Forest, western Kenya. It is a small, brown forest species formerly referred to *L. modestus*, but distinguished by differences in advertisement call and the sequence of the mitochondrial 16S rRNA gene. The specific allocation of certain related populations of *Leptopelis* in East and West Africa is briefly discussed.

Key words: Amphibia, bioacoustics, DNA, systematics, taxonomy

INTRODUCTION

The Kakamega Forest in western Kenya is an isolated forest remnant of the Guineo-Congolean rainforest belt. Its herpetofauna exhibits strong relationships with Central Africa (Köhler *et al.*, 2003). Currently, 27 anuran species are known from the Kakamega Forest and its vicinity (unpubl. data). Among them are two species of the genus *Leptopelis* Günther. Schiøtz (1975) tentatively referred the populations from Kakamega Forest to the terrestrial *Leptopelis bocagii* (Günther, 1864) and the arboreal *L. modestus* (Werner, 1898). The author noted morphological differences in the Kakamega Forest population of *L. bocagii*, which exhibits green instead of the brown dorsal coloration present in other areas. Schiøtz (1975) noted that the sample of *L. modestus* from Kakamega is very similar to *L. modestus* specimens collected by him in Obuda, Nigeria, as well as to specimens from the eastern Democratic Republic of Congo, which were allocated to *L. modestus* by Laurent (1973). Later, Schiøtz (1999) stated that the Nigerian populations are not conspecific with *L. modestus* and argued that East African populations may also be distinct, mainly because such an apparently discontinuous distribution pattern seems unlikely.

Leptopelis modestus was originally described from Cameroon by Werner (1898). The type locality was later restricted to "Buea, Cameroon" by lectotype designation (Perret, 1962). Perret (1962, 1966) considered *L. modestus* a forest species endemic to the southern Cameroon highlands. This view was adopted by Schiøtz (1999), who argued that the name *L. modestus* may refer to more than one species. However, at the same time, Schiøtz (1999) continued to use the name *L. modestus* for the populations from Nigeria and Kakamega Forest, Kenya.

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The confusing systematic status of East African *Leptopelis modestus*-like frogs led us to reinvestigate the status of the Kakamega Forest population. We concluded that it represents an unnamed species, which we describe here.

MATERIALS AND METHODS

Specimens examined are deposited at the National Museums of Kenya, Nairobi (NMK), the Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK) and the Zoologisches Museum Berlin (ZMB).

Field work was carried out in May and July 2004 at Buyangu Hill, northern Kakamega Forest (00°21'20" N, 34°52'40" E, ca. 1650 m above sea level). Adult specimens collected by us were preserved in 70% methanol; the single tadpole collected was stored in 4% formalin. A toe of one adult and the tail tip of the tadpole were clipped and stored in 98% ethanol for genetic analysis.

The terminology and description scheme follow those of Lötters *et al.* (2005). The webbing formula is according to Glaw & Vences (1994). Measurements are in millimetres and were taken as described by Lötters *et al.* (2005) with dial callipers to the nearest 0.1 mm. The snout-vent length is abbreviated SVL. Larval staging follows Gosner (1960). Tadpole body measurements were taken under a stereoscope.

Preserved tissue was used to sequence a 515 bp fragment of mitochondrial DNA, using the 16S rRNA gene; the allocation of the tadpole to the new species was confirmed by comparison of the respective DNA sequences. For methods and primers used see Lötters *et al.* (2004).

In July 2004, we recorded advertisement calls in the field from a chorus of males (recorded male not collected) using a digital Sharp MD-SR70 recorder and a Sennheiser Me-66 directional microphone. Air temperature at approximately 1.0 m above ground was measured with a Greisinger GFTH 95 immediately after recording. Recordings were sampled at a rate of

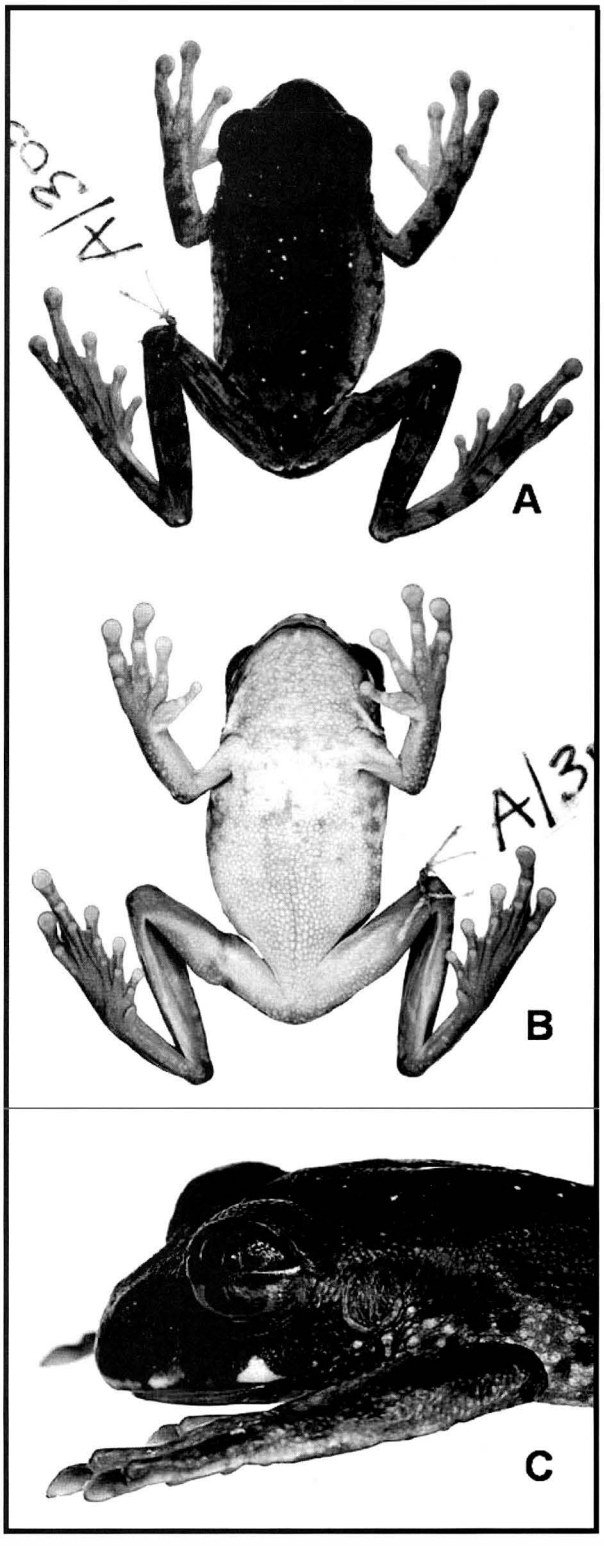

FIG. 1. Dorsal (A) and ventral views (B), as well as lateral view of head (C) of the preserved male holotype of *Leptopelis mackayi* sp. n. (NMK A/3057/1; SVL 34.0 mm). See Schiøtz (1999:271) for colour illustration of a live specimen of *L. mackayi* (as *L. modestus*).

22.05 kHz and 16-bit resolution and analysed with the sound analysis software Cool Edit 96 (Syntrillium Software Corp.). Frequency information was obtained through Fast Fourier Transformation (FFT, width 1024 points). Spectral settings in figures are Hanning window function with 256 bands resolution. Terminology in call descriptions follows Heyer *et al.* (1990) as extended by Köhler (2000).

SYSTEMATICS

LEPTOPELIS MACKAYI SP. N. (FIG. 1).

Leptopelis modestus (non Werner): Schiøtz, 1975:27 (partim); 1999:269 (partim).

Holotype. NMK A/3057/1, adult male, from Rondo Retreat Centre, Isecheno, 00°12'39" N, 34°46'36" E, 1550 m a.s.l., southern Kakamega Forest, Kakamega District, Western Province, Kenya; collected on 7 November 1994 by K. Howell.

Paratypes. NMK A/1407/1-2, adult and subadult males, NMK A/1407/3, adult female, from Bukura, Kakamega District, Western Province, Kenya, collected on 18 March 1983 by T. Madsen; ZFMK 83304-305, two adult males, from the northern slope of Buyangu Hill, northern Kakamega Forest, Kakamega District, Western Province, Kenya, collected on 18 and 30 May 2004 by B. A. Bwong.

Referred specimens. NMK A/3072/1, male (desiccated), from the Kakamega Forest (no precise locality data), Kakamega District, Western Province, Kenya, collected 1971 by A. Schiøtz; ZFMK 83306, tadpole in Stage 27, from the northern slope of Buyangu Hill, northern Kakamega Forest, Kakamega District, Western Province, Kenya, collected on 3 July 2004 by S. Schick and S. Lötters.

Diagnosis. A small arboreal *Leptopelis* with (1) adult males SVL 29–36 mm; adult female SVL about 40 mm; (2) head wider than long; (3) eye relatively large with horizontal eye diameter almost twice the distance from nostril to anterior corner of eye; (4) dorsal snout shape rounded; (5) tympanum distinct, its horizontal diameter slightly less than half the eye diameter; (6) dorsal skin finely granular, with small scattered tubercles; (7) feet one half webbed, hands one fourth webbed; (8) well-developed subarticular tubercles and terminal discs present on all toes and fingers; (9) pectoral glands present in males; (10) colour in life dorsally tan with brown markings; white fleck present below eye; laterally cream with small brown and whitish flecking; ventrally creamy white with few scattered brown flecks; iris bronze with fine black reticulation, eye periphery black; (11) vomerine odontophores distinct, forming two separate short rows, median between choanae; (12) sequence of a 515 bp fragment of the mitochondrial 16S rRNA gene as stored at GenBank (accession number AY940089).

Leptopelis mackayi is most similar to *L. modestus* from Cameroon to which it was formerly referred; the two differ in advertisement call characters and the sequence of the mitochondrial 16S rRNA gene (see below). Furthermore, *L. modestus* usually has lighter dorsal coloration and less tubercular dorsal skin compared to the new species. *Leptopelis fiziensis* Laurent, 1973, a taxon originally described as a subspecies of *L.*

modestus, differs from the new species in its slightly larger size, a somewhat more robust body and a grey vocal sac in life. *Leptopelis christyi* (Boulenger, 1912) from Uganda and the eastern Democratic Republic of Congo differs from the species described herein in its larger size, different dorsal colour pattern, white or yellow finger and toe discs in life and in advertisement call characters (Schiøtz, 1975, 1999; Köhler *et al.*, 2005). Other similar East African *Leptopelis* include *L. kivuensis* Ahl, 1929, and *L. karissimbensis* Ahl, 1929. The latter species inhabits montane grasslands and differs from *L. mackayi* in advertisement call characteristics (Schiøtz, 1975), a smaller tympanum and a more tuberculate dorsum. Like *L. mackayi*, *L. kivuensis* is an East African forest species. The latter mainly differs in having a white vocal sac and reddish iris in life as well as in advertisement call characteristics (Schiøtz, 1975, 1999). The enigmatic *L. fenestratus* Laurent, 1972, from the eastern Democratic Republic of Congo, differs from the new species in displaying full toe webbing (Laurent, 1972). The Central African *L. aubryi* (Duméril, 1856) is similar to *L. mackayi* in size and dorsal coloration, but differs from it in having a red or orange iris in life, smaller discs and in different characteristics of the advertisement call (Schiøtz, 1999). *L. omissus* Amiet, 1992 and *L. calcaratus* (Boulenger, 1906) might both be similar in size and coloration to *L. mackayi*, but differ from it in having a more pointed snout and a calcar on the heel (Amiet, 1991; Schiøtz, 1999).

Description of holotype. Adult male; body moderately robust, head slightly wider than body; snout rounded in dorsal view, rounded in lateral view; vomerine teeth present, in two short rows separated medially, median between choanae; choanae small, round; tongue longer than wide, cordiform, posterior third free; nostrils directed laterally, visible from dorsal view; canthus rostralis slightly curved; loreal region concave; eye relatively large with horizontal eye diameter almost twice the distance from nostril to anterior corner of eye; pupil vertical; tympanum distinct, round, its diameter less than half of eye diameter, tympanic annulus present; skin of all dorsal surfaces finely granular, with scattered small tubercles on dorsum; skin on ventral surfaces granular; pectoral glands present; hind limbs relatively long with tibia length reaching almost half SVL, tibiotarsal articulation reaching posterior corner of eye when hind limb adpressed; foot webbing formula 1(0), 2i(1) 2e(0), 3i(2) 3e(½), 4i(2) 4e(2), 5(½), all toes with lateral fringes; relative length of toes, I < II < III < V < IV; outer metatarsal tubercle indistinct, flat, small, inner well developed, ovoid; plantar surfaces strongly tubercular; well developed, round subarticular tubercles under all toes; tips of all toes bearing round discs, each about 1.5 times wider than width of adjacent phalange; hand webbing formula 1(1½), 2i(1½) 2e(1), 3i(2) 3e(1½), 4(1), all fingers with lateral fringes; relative length of fingers: I < II < IV < III, finger I < II when adpressed; outer metacarpal tubercle absent, inner weak, ovoid; palmar surfaces strongly tubercular; well developed, round subarticular tubercles under all fingers, with distal subarticular tubercles on fingers III and IV each slightly bifid; tips of all fingers bearing discs, each about 1.5 times wider than width of adjacent phalange. For measurements and ratios see Table 1.

In alcohol, dorsal surfaces tan with brown markings consisting of three diffuse transverse bars on forearm, four transverse bars on hind limb, small diffuse scattered flecks on dorsal surfaces of hands and feet, large hourglass pattern on dorsum with triangular blotch in interorbital region extending on to upper eyelid, and two transverse flecks in sacral region; 23 small cream-col-

TABLE 1. Measurements (in mm) and ratios of four adult males and one adult female of the type series of *Leptopelis mackayi* (NMK A/3057/1=holotype).

	NMK A/3057/1 (male)	NMK A/1407/1 (male)	ZFMK 83304 (male)	ZFMK 83305 (male)	NMK A1407/3 (female)
SVL	34.0	34.7	33.6	29.6	40.0
head width	12.7	13.9	13.0	11.2	15.6
head length	11.0	12.9	12.0	10.3	14.4
interorbital distance	5.2	4.1	4.5	4.0	5.2
eye diameter	4.9	5.3	5.5	4.6	6.1
tympanum diameter	2.3	2.5	2.2	2.0	2.9
eye–nostril distance	2.8	3.0	2.7	2.7	3.4
nostril–nostril distance	4.2	3.9	4.0	3.3	4.6
tibia length	16.9	17.4	17.1	16.3	22.0
foot length	14.8	16.2	15.1	13.5	18.1
hand length	11.1	11.2	10.9	9.4	12.8
head length/SVL	0.32	0.37	0.36	0.35	0.36
tibia length/SVL	0.50	0.50	0.51	0.55	0.55
headlength/head width	0.87	0.93	0.92	0.92	0.92
tympanum diameter/ eye diameter	0.47	0.47	0.40	0.43	0.48

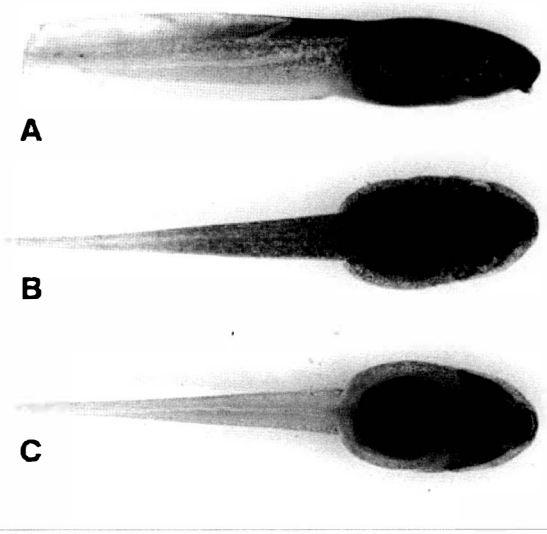

FIG. 2. Lateral (A), dorsal (B) and ventral (C) views (total length 22.4 mm) of tadpole of *Leptopelis mackayi* sp. n. (ZFMK 83306; Stage 27) (tail tip cut off for genetic analysis).

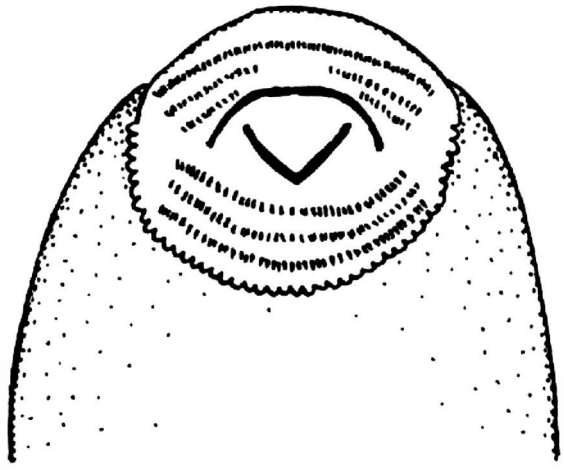

FIG. 3. Schematic drawing of labial tooth rows of tadpole of *Leptopelis mackayi* sp. n. (ZFMK 83306; Stage 27).

oured spots scattered on dorsum; tympanum tan, surrounded by brown; loreal region dark brown; tip of snout tan; creamy white fleck below eye; flanks tan with irregular brown and white flecks; supracloacal line cream, bordered with brown; all ventral surfaces cream with diffuse indistinct tan flecks on chest, throat and anterior half of belly.

Coloration in life differs only slightly from preserved specimens with ventral surfaces being bluish white and the vocal sac in calling males being blue. A living specimen of *L. mackayi* is figured in colour by Schiøtz (1999:271) under the name *L. modestus*.

Variation. The tympanum appears to be nearly round in most specimens of *L. mackayi*, but may be slightly oval in others. The brown dorsal markings can be more or less distinct. A distinct hour-glass pattern on the dorsum is missing in NMK A/1407/2. However, all specimens of *L. mackayi* exhibit brown flecks and markings. A dark triangular interorbital bar is present in five of the seven specimens. All specimens have a white fleck below the eye. Small creamy white dorsal spots are lacking in NMK A/1407/1-3. In the same specimens, the venter is uniform cream-coloured without brown spots or marbling. Brownish marbling on the throat is most distinct in the two males ZFMK 83304-305. In the same two specimens a white line on the heel is present. In NMK A/1407/1 and 3, the tan dorsum is covered by numerous small but prominent brown tubercles. For variation in measurements and ratios, see Table 1.

Tadpole. The specific allocation of a Stage 27 larva was verified by comparison of the sequence of a 515 bp fragment of the mitochondrial 16S rRNA gene with the respective sequence from adult *L. mackayi*. The larva has a total length of 22.4 mm (Figures 2 and 3; approximately 2 mm of tail tip cut off for tissue sampling), body elongated in dorsal view, ovoid, widest posterior to mid-body; snout truncated. Rounded eyes relatively large (diameter 0.8 mm), somewhat bulging, separated by distance slightly shorter than shortest distance from eye to tip of snout, positioned laterally and directed dorsolaterally, not visible in ventral view. Nares ovoid (horizontally elongated) and small, positioned dorsolaterally, slightly anterior to half the shortest distance from eye to tip of snout; visible in dorsal and lateral views. In lateral view, body highest at posterior half for about one third of body length; snout rounded. Spiracle sinistral, in length about half eye diameter; attached to body wall, positioned at somewhat anterior and below to half the shortest distance from eye and vent tube, and oriented posteriorly. Tail about two thirds of total length (tail length 14.4 mm), highest at anterior half for about half tail length (maximum tail height 3.1 mm). Caudal musculature equal in height from origin on the body up to half the length of the tail, from there gently decreasing. Fins moderate; dorsal fin

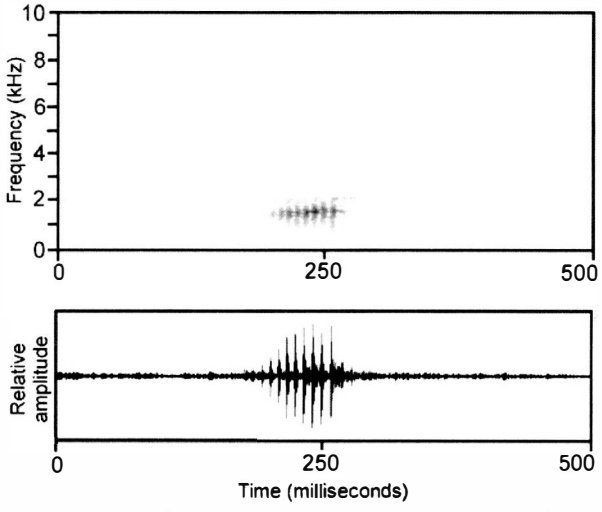

FIG. 4. Sound spectrogram and oscillogram of the advertisement call of *Leptopelis mackayi* sp. n., recorded on 2 July 2004 at Buyangu Hill, Kakamega Forest, Kenya; air temperature 15.0 °C.

not extending onto body, highest at mid-tail and posterior to it; ventral fin almost equally high up to slightly before tip of tail. Vent tube short, dextral, proximally linked to the caudal muscle, directed posteriorly. Oral disc not emarginated ventrolaterally, posteriorly and laterally bordered by a row of heavy, rounded marginal papillae. Submarginal papillae absent. Labial tooth row formula 3(2-3)/3; all rows almost equal in length, occupying almost the entire width of the oral disc. Jaw sheaths serrated, upper inversely U-shaped with a medial indentation, lower V-shaped and shorter.

In preservative, entirely light tan through scattered melanophores, most dense on dorsal surface of body; translucent on posterior venter; fins with fewest melanophores (uniformly arranged); melanophores absent on spiracle. Life coloration was brownish tan; eyes were black.

Few *Leptopelis* tadpoles have been described so far, and these seem not to exhibit great specific differences (e.g. Rödel, 2000; Channing, 2001). The tadpole of *L. mackayi* generally coincides with the characteristics given for the Leptopelinae by Altig & McDiarmid (1999).

Advertisement call. During field work in the Kakamega Forest area since 2001, calls of *L. mackayi* were frequently recognized, mainly during the period of major rainfall between April and July. They always sounded the same. We therefore suggest the vocalizations described below represent the advertisement call of *L. mackayi*. Males usually called from the canopy of small trees. Under these conditions, few individuals could be observed while calling. However, on 2 July 2004 a male (not collected) called in a chorus of conspecific males from a tree trunk approximately 4 m above the ground at the Buyangu area, northern Kakamega Forest. The call (Fig. 4) consists of a short pulsed note repeated at regular intervals and shows the following parameters (range followed by mean ± standard deviation in parentheses): note duration, 49–92 ms (77.50±15.96); pulses/note, 6–12 (9.80±1.99); pulses/second, 120–134 (127.79±5.06); notes/minute, 8.58–16.31 (13.19±2.43); frequency range, 350–1800 Hz; maximum call energy at 1507–1787 Hz (1590.56±81.66). Notes exhibit a moderate amplitude modulation reaching the maximum call energy in the second half of the note. Ten calls of one individual were analysed. The recording was obtained at 21.00 h after a light rain. Air temperature during recording was 15.0 °C.

Apart from advertisement calls, we recorded a single call consisting of a long pulsed note of 474 ms duration. The frequencies and pulse repetition rate of this long note are equal to those observed in short notes. Schiøtz (1975:28) figured a call from the Kakamega Forest, Kenya (as *L. modestus*), having a note duration of approximately 300–400 ms. This note appears to be similar to the single long note recorded by us. Schiøtz (1975, 1999) termed this call a "buzzing call" and doubted that it is a true mating call given the very low calling activity observed by him. Schiøtz's (1975, 1999) interpretation is in accordance with our observations, since during regular calling activity we always observed the short notes described as advertisement calls above. The long note figured by Schiøtz (1975) and recorded by us may therefore have a territorial function. Such different call types have already been documented for other species of *Leptopelis* (e.g. Grafe *et al.*, 2000).

Amiet & Schiøtz (1974) and Schiøtz (1999) provided a figure of a call of *L. modestus* from Ongot, Cameroon, which most probably corresponds to the true *L. modestus*. Although numerical parameters are lacking, it is obvious from the figure that the note duration is considerably longer and pulse repetition rate higher compared to the call of *L. mackayi*. In addition, the notes of *L. modestus* are repeated at much shorter intervals, sometimes given as two-note calls. All these characters are lacking in calls reported for *L. mackayi*.

Molecular genetics. The sequence of a 515 bp fragment of the mitochondrial 16S rRNA gene obtained from ZFMK 83304 has been stored at GenBank (accession number AY940089). Comparison of this sequence with the respective one of *L. modestus* from Kodmin, Bakossi Mountains, Cameroon (ZFMK 67976, GenBank accession number AJ437013) revealed that they are less closely related than their morphological similarities would suggest. The samples compared differed in 41 bp (38 substitutions, 3 gaps), which equals a substitution level of about 8 %.

Natural history. The Kakamega Forest area contains highland rain forest at 1500–1700 m a.s.l. Annual precipitation ranges between 1500 and 2000 mm with bimodal rainfall maxima from April to July and September to October. So far, *L. mackayi* is only known from forest habitats. Although this species survives in secondary and disturbed forest, further logging activities may threaten its survival. Because males were heard calling between April and September, i.e. during the rainy season, we assume this period to correspond to the breeding season of *L. mackayi*.

The single tadpole was found in a water-filled puddle with a surface area less than 0.5 by 0.5 m and a depth of approximately 0.2 m in secondary forest. This puddle was part of a very small cascading stream with numerous puddles of different sizes. It seemed that these puddles were recently produced by fast-flowing water, because they did not contain any aquatic vegetation and few organic materials. We were not able to trace additional larvae in the same or nearby puddles. Although males of *L. mackayi* were calling from trees just above this small stream, it seems unlikely that the puddle is equivalent to the oviposition site. More probably, eggs are deposited in the soil and at hatching tadpoles wriggle over the ground to the water to continue their development.

Distribution. The new species is known only from the Kakamega Forest and its vicinity in western Kenya. We will not rule out that it also occurs in adjacent

Uganda and possibly the eastern Democratic Republic of the Congo (see Discussion).

Etymology. The specific name is a patronym for the late Alex Duff-MacKay, curator of Herpetology at the National Museums of Kenya from 1972 to 1995, and one of the pioneer explorers of the East African amphibian fauna.

DISCUSSION

Despite similarities in morphology, Schiøtz (1975; 1999) argued that East African populations and those from Cameroon and Nigeria referred to *Leptopelis modestus* may not be conspecific and that this name possibly includes a complex of cryptic species. We have demonstrated that the Kakamega Forest population tentatively referred to *L. modestus* by Schiøtz (1975; 1999) actually represents a distinct species. The genetic and bioacoustic differences from Cameroonian specimens are obvious.

Laurent (1973) and Schiøtz (1975) assigned East African populations from the eastern Democratic Republic of Congo to *L. modestus*. It seems possible that these populations may also represent *L. mackayi*. However, the genetic and bioacoustic data necessary to verify this hypothesis are lacking and thus the taxonomic status of these populations remains unclear. Due to the close relationship of the anuran fauna of the Kakamega Forest to more western forests, *L. mackayi* may also occur in adjacent Uganda.

Schiøtz (1967) reported *L. modestus* from the Obudu Plateau, Nigeria, but noted that this identification is probably doubtful. Subsequently, Amiet & Schiøtz (1974) and Schiøtz (1975) referred to Nigerian populations as *L. modestus*, whereas Schiøtz (1999) stated that the populations from the Obudu Plateau and high altitudes in Cameroon represent a distinct undescribed form. The dorsal colour pattern exhibited by the preserved specimen from Nigeria figured by Schiøtz (1967:54) is rather similar to that of *L. mackayi*. Furthermore, the figured call of the Obudu population (Schiøtz, 1967:56) appears to be similar to that of *L. mackayi* in duration, pulse rate and frequency. Due to the geographical distance between eastern Nigeria and western Kenya, it seems improbable that Nigerian populations and *L. mackayi* are conspecific. Nevertheless, when referring to the advertisement calls, it is clear that the calls of *L. mackayi* and of populations from eastern Nigeria are both different from calls of *L. modestus* from Cameroon. Although this does not necessarily imply specific distinctness, it is at least highly unlikely that all populations mentioned above are conspecific, especially in light of the great distances between them.

The hitherto unsuspected distinctness of East African and West African populations of the species pair, *Leptopelis modestus* and *L. mackayi* may also hold true for other species complexes currently considered to represent a single widespread taxon. For example, Amiet (2004) recorded two species of *Leptopelis* from Cameroon, which he referred to as *L.* cf. *bocagii* and *L.* cf. *christyi*. In both cases, the author pointed out various differences in populations formerly known of these species. The type locality of *L. christyi* is situated in Uganda, East Africa. Call recordings of *L. christyi* from Uganda (Köhler *et al.*, 2005) differ considerably from Cameroonian call recordings by Amiet (2004) and thus may argue for genetic differentiation of mentioned populations. Concerning sub-Saharan distribution patterns of anurans, an east-west divide at species group level might be more strongly pronounced than currently documented (compare Poynton, 1999). Further studies may possibly discover the presence of more diverse and more endemic anuran faunas in East and West Africa respectively.

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REFERENCES

Altig, R. & McDiarmid, R. W. (1999). Diversity. Familial and generic characterizations. In *Tadpoles: The Biology of Anuran Larvae,* 295–337. McDiarmid, R. W. & Altig, R. (Eds.). Chicago and London: University of Chicago Press.

Amiet, J.-L. (1991). Un *Leptopelis* méconnu de la faune forestière camerounaise (Amphibia, Anura, Hyperoliidae). *Alytes* **9**, 89–102.

Amiet, J.-L. (2004). A propos de deux *Leptopelis* nouveaux pour la faune du Cameroun (Anura, Hyperoliidae). *Alytes* **21**, 111–170.

Amiet, J.-L. & Schiøtz, A. (1974). Voix d'amphibiens camerunais III. Hyperoliidae: genre *Leptopelis*. *Annales de la Faculté des Sciences du Cameroun* **17**, 131–163.

Channing, A. (2001). *Amphibians of Central and Southern Africa.* Ithaca and London: Cornell University Press.

Glaw, F. & Vences, M. (1994). *A Fieldguide to the Amphibians and Reptiles of Madagascar*, Second edition. Cologne: Vences & Glaw.

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

Grafe, T. U., Steffen, J. O. & Stoll, C. (2000). Vocal repertoire and effect of advertisement call intensity on

calling behavior in the West African tree frog, *Leptopelis viridis*. *Amphibia–Reptilia* **21**, 13–23.

Heyer, W. R., Rand, A. S., da Cruz, C. A. G., Peixoto, O. L. & Nelson, C. E. (1990). Frogs of Boracéia. *Arquivos de Zoologia* **31**, 237–410.

Köhler, J. (2000). Amphibian diversity in Bolivia: a study with special reference to montane forest regions. *Bonner Zoologische Monographien* **48**, 1–243.

Köhler, J., Wagner, P., Visser, S. & Böhme, W. (2003). New country records of *Adolfus africanus* (Sauria: Lacertidae) – a rainforest lizard with disjunct distribution? *Salamandra* **39**, 241–248.

Köhler, J., Scheelke, K., Schick, S., Veith, M. & Lötters, S. (2005). Contribution to the taxonomy of hyperoliid frogs (Amphibia: Anura: Hyperoliidae): advertisement calls of twelve species from East and Central Africa. *African Zoology* **40**, 127–142.

Laurent, R. F. (1972). Amphibiens. *Exploration du Parc National des Virunga, 2e Série* **22**, 1–125.

Laurent, R. F. (1973). Le genre *Leptopelis* Günther (Salientia) au Zaire. *Musée royal d'Afrique Centrale, Série IN-8, Science zoologique* **202**, 1–62.

Lötters, S., Rotich, D., Scheelke, K., Schick, S., Teege, P., Kosuch, J. & Veith, M. (2004): Bio-sketches and partitioning of syntopic reed frogs, genus *Hyperolius* (Amphibia: Hyperoliidae), in two humid tropical African forest regions. *Journal of Natural History* **38**, 1969–1997.

Lötters, S., Rödel, M.-O. & Burger, M. (2005). New large tree frog from north-western Gabon (Hyperoliidae: *Leptopelis*). *Herpetological Journal* **15**, 149–152.

Perret, J.-L. (1962). Révision des types de *Leptopelis* et note sur quelques *Hyperolius* (Amphibia Salientia) de la région camerounaise, conservés au Museum de Berlin. *Revue zoologique botanique africaine* **64**, 235–246.

Perret, J.-L. (1966). Les amphibiens du Cameroun. *Zoologisches Jahrbuch (Systematik)* **8**, 289–464.

Poynton, J. C. (1999). Distribution of amphibians in sub-Saharan Africa, Madagascar, and Seychelles. In *Patterns of distribution of amphibians. A global perspective,* 483–539. Duellman, W. E. (Ed.). Baltimore and London: Johns Hopkins.

Rödel, M.-O. (2000). *Herpetofauna of West Africa, Vol. I: Amphibians of the West African savanna*. Chimaira, Frankfurt am Main.

Schiøtz, A. (1967). The treefrogs (Rhacophoridae) of West Africa. *Spolia Zoologica Musei Hauniensis* **25**, 1–346.

Schiøtz, A. (1975). *The Treefrogs of Eastern Africa*. Copenhagen: Steentrupia.

Schiøtz, A. (1999). *Treefrogs of Africa*. Frankfurt/Main: Chimaira.

Werner, F. (1898). Über Reptilien und Batrachier aus Togoland, Kamerun und Tunis II. *Verhandlungen der zoologisch-botanischen Gesellschaft Wien* **48**, 191-231.

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APPENDIX 1

SPECIMENS EXAMINED FOR COMPARISONS

Leptopelis aubryi (12 specimens): GABON: Cap Esterias, ZFMK 73257-258; Barrage de Tchimbélé, ZFMK 73156-157, 73176-177, 73279; Barrage de Kinguélé, ZFMK 73197; Lambaréné, ZFMK 73127-128; Fougamou, ZFMK 73235-236.

Leptopelis christyi (14 specimens): UGANDA: Semliki National Park: Mt. Ruwenzori, ZFMK 63115, Ntandi, ZFMK 66483; Budongo Forest, ZFMK 64207-212.

Leptopelis fiziensis (18 specimens): DEMOCRATIC REPUBLIC OF CONGO: S-Kivu, Kahuzi-Biega, ZFMK 63935; RWANDA: Nyungwe Forest: Mukina (Kitabi), ZFMK 58769-770, 58798-800, Rwasenkoko, ZFMK 58783, Cyamudongo, ZFMK 63912-917; Préf. Gikongoro, Kwagahunga, ZFMK 58687, Mugatemba, ZFMK 58750-751; Gishwati, Gikunbu, ZFMK 63918; UGANDA: Mt. Ruwenzori, Nyakalengijo, ZFMK 63184.

Leptopelis karissimbiensis (4 specimens): RWANDA: NW Gishwati, ZFMK 63919-922.

Leptopelis kivuensis (1 specimen): RWANDA: Kissenje, ZMB 25324 (holotype).

Leptopelis modestus (80 specimens): CAMEROON: Buea, ZMB 28708 (lectotype), 66638 (paralectotype); Kodmin, Bakossi Mountains, 1345 m a.s.l., ZFMK 64408-420, 67922-978; Nguengué, Mt. Nlonako, ZFMK 75456; without precise locality, ZMB 14112, 200046, 66637, 66639-340 (all paralectotypes); EQUATORIAL GUINEA: Makomo, ZMB 20051, 66636 (syntypes of *Hylamabtes rufus* var. *ventrimaculata*).

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SYSTEMATICS OF THE NOSE-HORNED VIPER (*VIPERA AMMODYTES*, LINNAEUS, 1758)

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Geographic variability of the nose-horned viper (*Vipera ammodytes*) was analysed using multivariate techniques in order to clarify the taxonomic status and geographic ranges of the subspecies. Analyses included samples ranging from central northern Italy and southern Austria to easternmost Turkey, Georgia and Armenia, and hence, all described taxa. In total, 14 morphometric, five meristic and nine qualitative traits of 922 specimens (451 males and 471 females) were recorded and analysed using different multivariate statistics. The results showed the validity of four subspecies: one inhabiting the western and central parts of the species' range (from Italy, via Austria, to the western and central parts of the Balkans), the second occurring in the southernmost part of the Balkan peninsula, the third distributed from the southern and eastern Balkans to western Turkey and the fourth inhabiting eastern Turkey, Georgia and Armenia.

Key words: Balkans, multivariate analyses, snake, subspecies

INTRODUCTION

The nose-horned viper (*Vipera ammodytes*, L. 1758) is one of the most widespread vipers of southern Europe. Its range extends from central northern Italy, southern Austria, through the Balkans and southern Romania to north-eastern Turkey and Transcaucasia (Arnold & Ovenden, 2002). According to some of the relevant literature (Eiselt & Baran, 1970; Biella, 1983; Biella & Blättler, 1989; Crnobrnja-Isailovic & Haxhiu, 1997) seven different subspecies have been described so far: *V. a. ammodytes*, Linnaeus, 1758; *V. a. illyrica*, Laurenti, 1768; *V. a. meridionalis*, Boulenger, 1903; *V. a. montandoni*, Boulenger, 1904; *V. a. transcaucasiana*, Boulenger, 1913; *V. a. ruffoi*, Bruno, 1968; and *V. a. gregorwallneri*, Sochurek, 1974. *V. a. transcaucasiana* is sometimes treated as a full species (Obst, 1983; Nilson *et al.*, 1999). However, the taxonomic status of some subspecies (*V. a. illyrica*, *V. a. ruffoi*, *V. a. gregorwallneri* and even *V. a. montandoni*) remains unclear. Generally, *V. a. illyrica* is treated as a synonym of the nominate subspecies (see Tomovic & Dzukic, 2003 and references therein). Golay *et al.* (1993) recognized only three subspecies: *V. a. ammodytes* (including *gregorwallneri* and *ruffoi*), *meridionalis* (including *montandoni*) and *transcaucasiana*. In contrast, Ulber (1994) recognized five subspecies: *V. a. ammodytes*, *meridionalis*, *montandoni*, *transcaucasiana* and *gregorwallneri*. In some of the latest references (e.g. McDiarmid *et al.*, 1999) only synonyms are listed and the taxonomic status of the subspecies is not considered. Although this species has been the subject of intense interest concerning its systematics and distribution several times in the past century (Fuhn & Vancea, 1961; Vozenilek, 1971; Sochurek, 1972, 1976, 1985; Vozenilek & Cizek, 1978; Biella, 1983; Biella & Blättler, 1989; Brodmann, 1987), comprehensive studies using multivariate statistics to define subspecies from the whole species range have never been conducted. Since a previous study of *Vipera ammodytes* (Tomovic & Dzukic, 2003) had been carried out on populations from the central and eastern parts of the species' range only and had failed to show clear differentiation of the subspecies, additional analyses of populations from the rest of the range were needed for a complete taxonomic consideration of the nose-horned vipers.

Multivariate statistics were used to define taxonomic units up to the species level, as has recently been done by numerous authors for different snake taxa (Wüster & Thorpe, 1987; Wüster *et al.*, 1995; Wüster & Broadley, 2003; Zuffi & Bonnet, 1999; Zuffi, 2002). The advantage of these techniques is that they analyse the pattern of variation in all characters used simultaneously (Thorpe, 1987; Wüster *et al.*, 1992).

The aims of this study were: (1) to elucidate the pattern of geographic variation in the nose-horned viper across its entire range; (2) to test the validity of the conventional subspecies; (3) to take into consideration possible geographic ranges of re-defined subspecies.

MATERIALS AND METHODS

STUDY AREA AND SAMPLES

The study area includes the whole range of the species, from central northern Italy and southern Austria, through the Balkans to Turkey, Georgia and Armenia (Fig. 1). I examined preserved specimens from the collections of the Naturhistorisches Museum in Vienna; the Museum of Natural History in Ljubljana; the Land Museum – Natural History Department in Sarajevo; the Institute for Biological Research in Belgrade; the Museum of Natural History in Belgrade; the Museum of Natural History in Skoplje; the Institute of Biology,

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1-NC Italy (N=2,2)

2-S Austria, Slovenia, NE Italy, NW Croatia, N Dalmatia, NW B&H (N=100,111)

3-C Dalmatia, C B&H (N=26,31)

4-S Dalmatia, SE Bosnia & Herzegovina, Montengro (N=82,95)

5-Serbia, SW Romania, NW Bulgaria, NW Macedonia, N Albania (N=84,94)

6-Bulgaria (except NW), Macedonia (except NW), Serbia (S part), E Greece, C Albania, NW Turkey (N=77,84)

7-C Greece, Peloponnesus, Cyclades Islands (N=24,29)

8-E Turkey, Georgia, Armenia (N=5,5)

FIG. 1. Map of geographic groups analysed. Numbers of specimens per group of males are given in parentheses (first number = sample size used for analyses of morphometric characters, second number = sample size used for analyses of qualitative traits).

University of Priština; the National Museum of the Bulgarian Academy of Sciences in Sofia; and the private collections of I. Krizmanic, R. Ajtic, J. Crnobrnja-Isailovic and myself.

In total, 922 specimens (451 males and 471 females) were analysed. For morphometric analyses, only adult and subadult animals (799 specimens – 400 males and 399 females) were examined.

Since the number of specimens per locality was insufficient for performing statistical analyses, several localities were a priori pooled into compound geographic groups, based on collecting gaps and

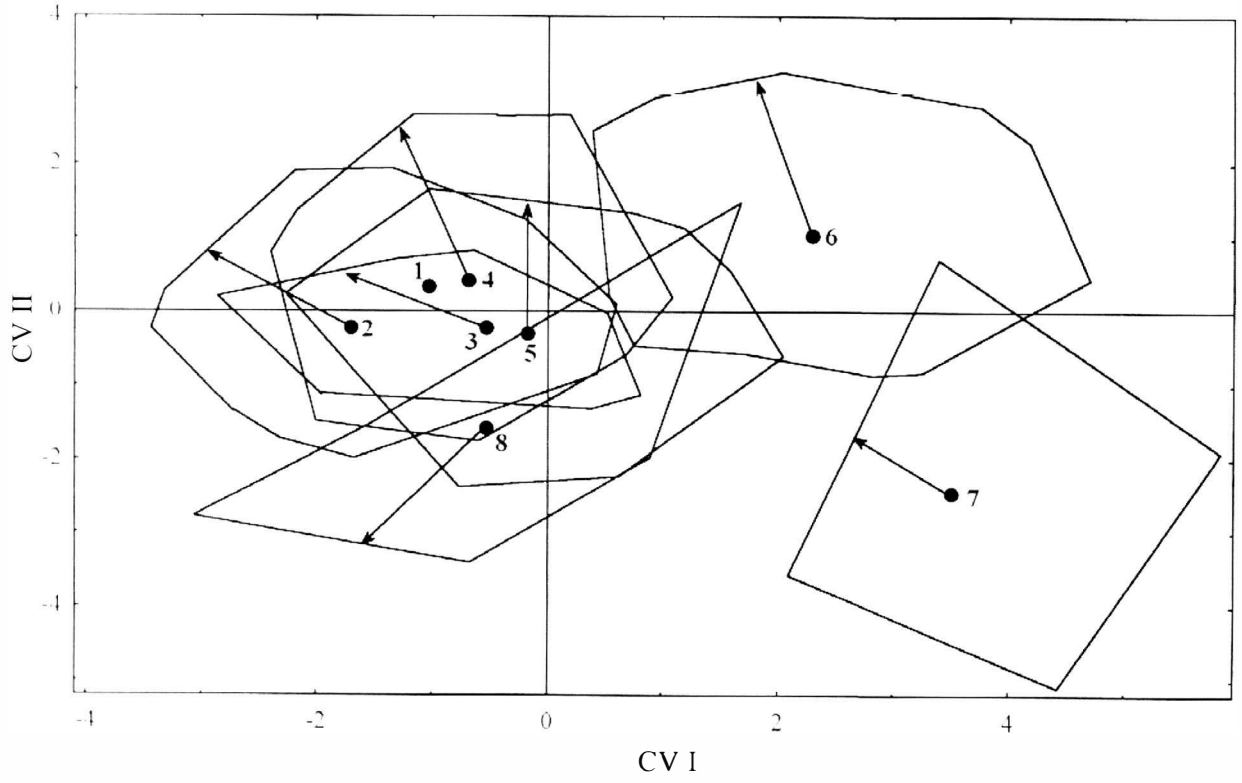

FIG. 2. Relative position of individual male specimens in the projection of the first and the second canonical variates (size-adjusted morphometric and meristic data). Points denote means of geographic groups, lines indicate the position of far-distant individual specimens (outliers were removed upon 95% confidence level).

TABLE 1. Character coefficients for three canonical variates from the discriminant function analysis of the nine groups of males. Significant coefficients are in bold.

Character	CV I	CV II	CV III
Lt cor	0.050	0.009	0.163
Alt cor	-0.385	-0.126	-0.142
L cd	-0.219	-0.038	-0.122
L cap	-0.165	0.223	-0.004
Lt cap	0.174	0.344	**0.587**
Alt cap	0.058	0.125	0.216
D o	0.247	0.201	-0.440
Dols	-0.183	0.370	-0.455
Alt corni	0.118	**-0.553**	-0.065
Alt r	-0.082	-0.329	0.194
L scr	**0.612**	0.404	-0.247
Lt scr	-0.463	-0.254	0.166
D	-0.110	0.064	0.370
V	**-0.856**	0.446	-0.362
S	0.059	0.290	**0.619**
Eigenvalue	2.375	0.699	0.431
Cum. prop.	60.2%	77.9%	88.8%

physiographic barriers. By using principal component analysis (PCA) the groups were tested for geographic heterogeneity. If analyses showed heterogeneity of the samples they were split into separate groups, and if the variability of the samples overlapped, they were pooled together.

TABLE 2. Mean values, standard deviations and ranges of significant discriminant morphometric (size-adjusted data) and meristic (original data) characters for the four re-defined subspecies (males).

	Mean	Min	Max	SD
V.a.ammodytes (n=294)				
Lt cap	17.9	11.8	23.0	1.69
Alt corni	4.1	2.6	6.6	0.57
L scr	3.3	2.2	4.5	0.37
V	152.0	139.0	161.0	4.13
S	35.9	25.0	45.0	2.78
V.a.meridionalis (n=24)				
Lt cap	17.9	12.7	21.8	2.14
Alt corni	4.3	3.2	5.3	0.56
L scr	3.6	3.0	4.2	0.38
V	138.8	130.0	150.0	4.57
S	31.4	23.0	34.0	2.60
V.a.montandoni (n=77)				
Lt cap	19.2	15.0	23.5	1.54
Alt corni	3.6	2.3	5.4	0.68
L scr	4.0	3.1	4.8	0.39
V	146.8	137.0	156.0	4.23
S	34.8	30.0	38.0	1.87
V.a.transcaucasiana (n=5)				
Lt cap	16.1	15.6	16.7	0.44
Alt corni	4.5	2.8	5.5	1.06
L scr	3.0	2.4	4.3	0.76
V	151.2	145.0	156.0	4.32
S	36.0	33.0	38.0	2.12

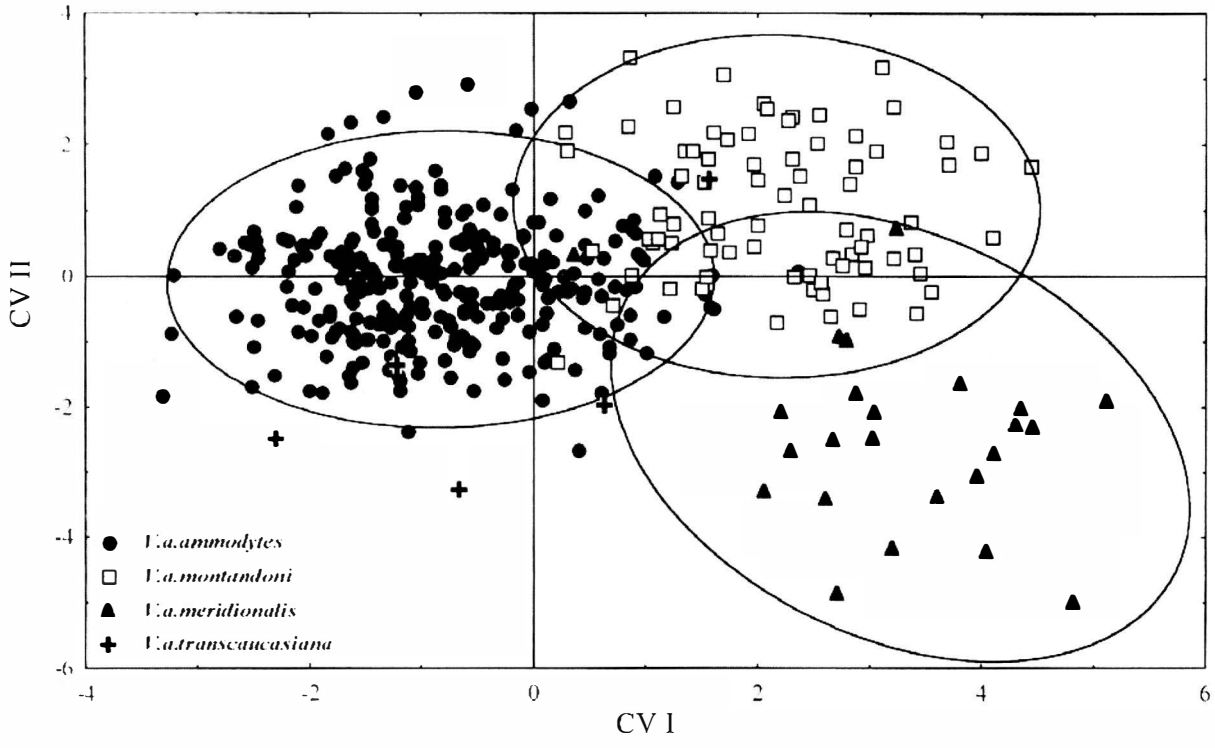

FIG. 3. Relative position of individual male specimens of re-defined subspecies in the projection of the first and the second canonical variates (size-adjusted morphometric and meristic data). Lines indicate 95% confidence level.

TABLE 3. Results of *post hoc* tests (Spjotvoll/Stoline test for unequal *n*) of comparison of means for significant discriminant morphometric (size-adjusted data) and meristic (original data) characters for the four redefined subspecies (males). n.s., $P>0.05$; *$P<0.05$; **$P<0.01$; ***$P<0.001$.

	V. a. ammodytes	*V. a. meridionalis*	*V. a. montandoni*
Lt cap			
V. a. meridionalis	n.s.		
V. a. montandoni	***	*	
V. a. transcaucasiana	n.s.	n.s.	*
Alt corni			
V. a. meridionalis	n.s.		
V. a. montandoni	***	**	
V. a. transcaucasiana	n.s.	n.s.	n.s.
L scr			
V. a. meridionalis	**		
V. a. montandoni	***	***	
V. a. transcaucasiana	n.s.	n.s.	***
V			
V. a. meridionalis	***		
V. a. montandoni	***	***	
V. a. transcaucasiana	n.s.	***	n.s.
S			
V. a. meridionalis	***		
V. a. montandoni	*	***	
V. a. transcaucasiana	n.s.	*	n.s.

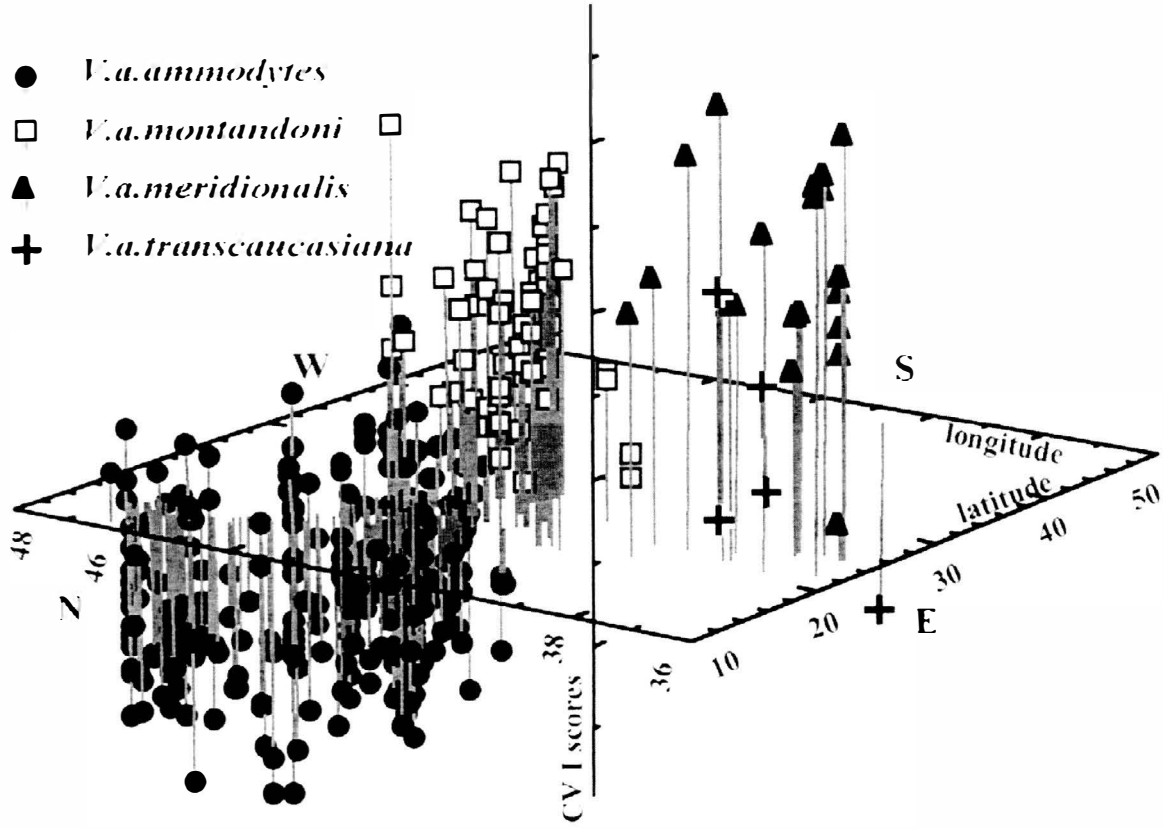

FIG. 4. Three-dimensional scatter plot of individual CV I scores against geographic positions (longitude and latitude) for the redefined taxa.

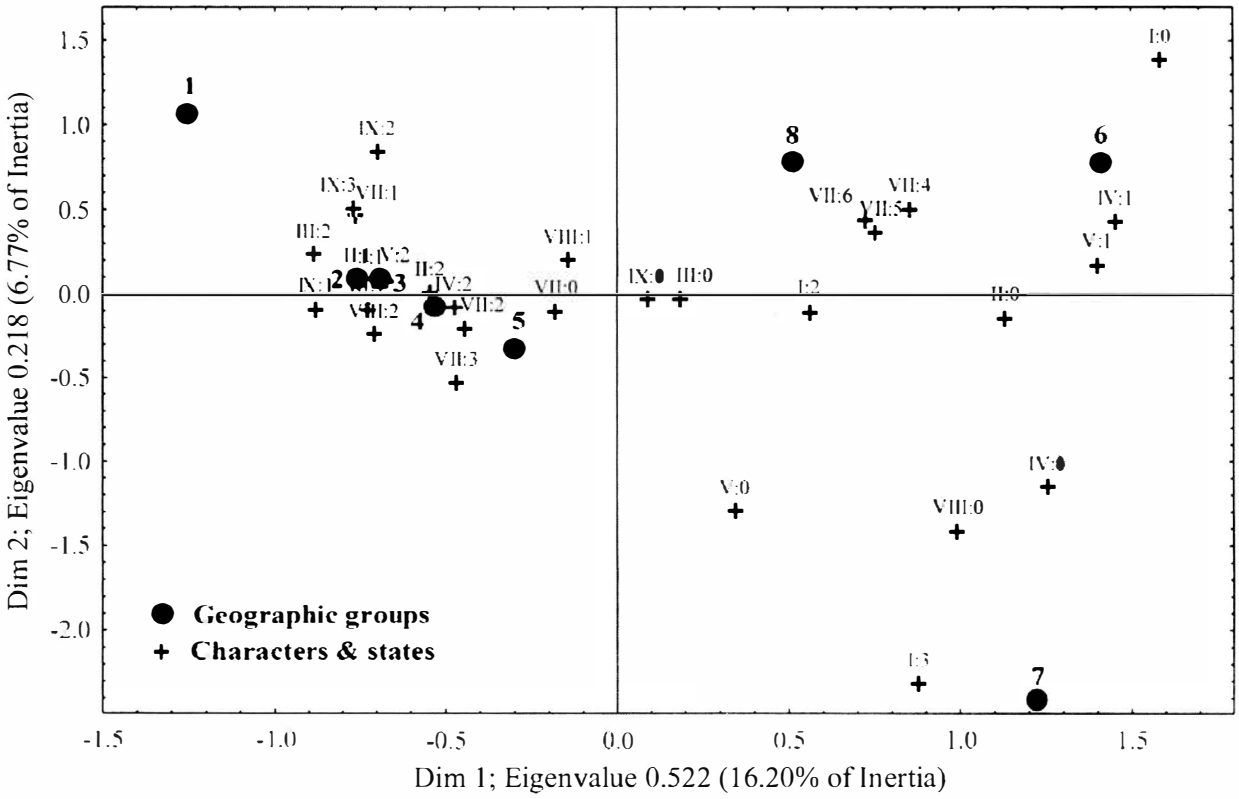

FIG. 5. Scatter-plot of coordinates of the columns (geographic group) and rows (character states) on the first and the second correspondence axes (qualitative data), for males.

CHARACTERS

Morphometric traits. For each specimen, 14 morphometric measures were recorded with a string (to 1 mm precision) and with digital callipers (to 0.01 mm precision): SVL – snout-vent length; L cor – body length (without head); Lt cor – body width (at mid-body point); Alt cor – body height (at mid-body point); L cd – tail length; L cap – head length (from the tip of the snout to the articulation point of the lower jaw and quadrate); Lt cap – head width (across the widest part of the head); Alt cap – head height (at the highest point of the head); D o – eye diameter (mean value of the both sides); Dols – distance between the eye and upper labial (mean value of the both sides); Alt corni – horn height (from the rostral plate to the top of the horn); Alt r – snout height (from edge of the upper lip to the canthus rostralis); L scr – height of rostral plate; Lt scr – width of rostral plate.

Meristic traits. Five meristic characters were counted: number of dorsal scale rows (D), number of ventral (V) and subcaudal scales (S), number of gular scales (G) and number of sublabials contacting one inframaxillar scale (IM/SL).

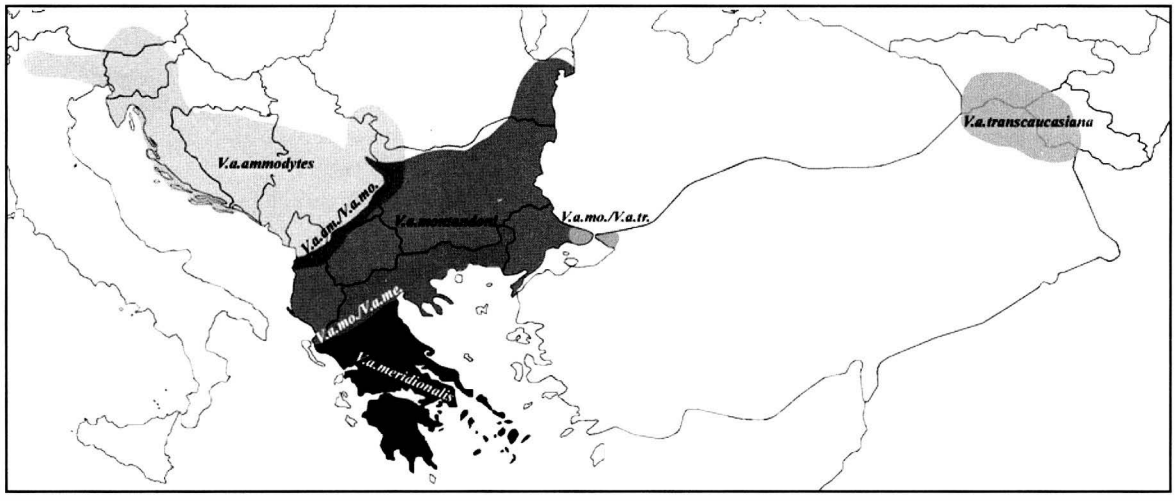

FIG. 6. Distribution ranges of redefined subspecies and transition zones.

TABLE 5. Frequencies of qualitative character states of re-defined subspecies (males).

	V. a. ammodytes	*V. a. meridionalis*	*V. a. montandoni*	*V. a. transcaucasiana*
I:0	3	1	32	1
I:1	238	1	5	4
I:2	75	14	42	0
I:3	17	13	5	0
II:0	58	28	83	5
II:1	244	0	0	0
II:2	31	1	1	0
III:0	183	29	83	4
III:1	33	0	1	0
III:2	33	0	0	1
IV:0	9	4	6	0
IV:1	8	15	58	1
IV:2	316	10	20	4
V:0	35	7	7	0
V:1	15	21	75	2
V:2	283	1	2	3
VI:0	19	0	0	0
VI:1	12	0	3	0
VI:2	6	3	1	0
VI:3	151	15	48	0
VI:4	79	4	0	0
VI:5	60	5	23	0
VI:6	6	2	9	0
VI:7	0	0	0	5
VII:0	25	0	3	0
VII:1	39	0	1	0
VII:2	75	4	4	0
VII:3	135	14	1	0
VII:4	21	4	27	4
VII:5	34	7	43	0
VII:6	4	0	5	1
VIII:0	15	20	15	1
VIII:1	315	9	69	4
VIII:2	3	0	0	0
IX:0	290	28	82	5
IX:1	23	0	0	0
IX:2	3	0	0	0
IX:3	17	1	2	0
Total	333	29	84	5

Qualitative traits. For analysis of qualitative traits, I used the following nine, with taxonomic significance (see Tomovic & Dzukic, 2003 and references therein): I – number of suprarostral plates (with states 0: suprarostral lacking – joined with rostral plate, 1: one suprarostral plate, 2: two suprarostral plates, 3: three suprarostral plates); II – connection of nasorostral plates with canthus rostralis (with states 0: neither nasorostral plate in contact with canthus rostralis, 1: both nasorostral plates in contact with canthus rostralis, 2: asymmetry – one nasorostral plate in contact with canthus rostralis, the other not); III – nasorostral plates (with states 0: undivided, 1: one plate divided, 2: both plates divided); IV – rostral height/width ratio (with states 0: equal height and width, 1: greater height, 2: greater width); V – the relation between the rostral and nasorostral plates (with states 0: heights of rostral and nasorostral plates equal, 1: height of rostral plate greater, 2: height of nasorostral plates greater); VI – type of dorsal trunk pattern (with types 0, 1, 2, 3, 4, 5, 6, 7; see Tomovic & Dzukic, 2003, Fig. 2A); VII – type of head pattern (with types 0, 1, 2, 3, 4, 5, 6; see Tomovic & Dzukic, 2003, Fig. 2B); VIII – the type of lower lip spots (with states 0: undivided spot, 1: divided spot, 2: spot lacking); and IX – presence of large scales on the dorsal side of the head (with states 0: large scales absent, 1: parietal present, 2: frontal present, 3: both parietal and frontal scales present). Since scoring of characters VI and VII could be subjective, I compared variability of all specimens first and then defined the categories. The colour of the ventral surface of the end of the tail was considered a significant taxonomic character among nose-horned vipers. In this study, this character could not be scored due to the poor condition of older alcohol-preserved specimens.

STATISTICAL METHODS

Both morphometric and meristic characters were checked for between-group differences by means of ANOVA (meristic) and ANCOVA (morphometric, with SVL as the independent variable) and only those that displayed significant between-group differences (15 characters) were then used for multivariate analyses. In order to assess the variation across the geographic groups free of the effects of body size, each individual's linear measurement was regressed to the mean snout-vent length of 502 mm, using the pooled within-group regression coefficient obtained by ANCOVA. Discriminant analyses were run on meristic traits and size-adjusted morphometric data together. Since the sexual dimorphism of morphological traits had previously been confirmed (Tomovic *et al.*, 2002), analyses for males and females were done separately. Due to similar discrimination patterns between the sexes, the results are given for males only.

Discriminant function analysis was performed on size-adjusted morphometric and meristic data, in order to clarify the relative importance of characters as discriminators between a priori groups and the relative positions of the centroids and individual specimens of geographic groups (Manly, 1986). In order to avoid pooling of specimens from the possible intergradation zones, they were excluded from the canonical analyses of pre-defined geographic groups consisting of unambiguous specimens; the analysis placed specimens from possible intergradation zones without any a priori assignment to any geographic group. Those specimens were entered into the analyses after the canonical variates/discriminant functions had been established and were placed into previously defined groups a posteriori. Thus, eight final geographic groups were established (Fig. 1).

With the results of the multivariate techniques, subspecies were re-defined and univariate statistics (descriptive statistics and *post hoc* Spjotvoll/Stoline test

for unequal *n*) were employed only for the characters that significantly contributed to discrimination of the samples. Mean values, ranges and standard deviations of discriminative morphometric and meristic characters for the re-defined subspecies are given for comparison with previously published data (Table 2). Also, the results of *post hoc* tests (Table 3) were applied in order to evaluate the taxonomic value of previously used characters for identification of re-defined subspecies.

Correspondence analysis (Rohlf, 1988) was used in order to clarify which qualitative characters (and states) define the taxonomic units. The output of such an analysis was the coordinates of the row (subspecies) and column (character states) on correspondence axes displayed on the scatter plot. Frequencies of qualitative traits of re-defined taxa are given in Table 5 (note: for character III, some specimens were not included in analyses due to lack of data).

Statistical analyses were performed with statistical package Statistica (version 5.1) for Windows 95 (StatSoft Inc., 1997).

RESULTS

Morphometric and Meristic Data

Discriminant function analysis. Discriminant function analysis on size-adjusted morphometric and meristic data of males showed that five characters – height of rostral plate (L scr), number of ventral plates (V), height of horn (Alt corni), width of head (Lt cap) and number of subcaudal plates (S) – contributed significantly to the discrimination of geographic groups (Table 1). The first discriminant function canonical variate clearly separated the southern and eastern groups from the groups inhabiting the central and north-western parts of the Balkan peninsula (Fig. 2). The second discriminant function canonical variate additionally separated the samples from the southernmost part of the range (central Greece with Peloponnesus and Cyclades Islands) from the groups inhabiting the eastern and southern parts of the Balkans (central Albania, southern Serbia, Former Yugoslav Republic of Macedonia (excluding NW part), Bulgaria (excluding NW part), SE Romania, E Greece and NW Turkey). The geographic group from the easternmost part of the range (8 – E Turkey, Georgia, Armenia) was also separated from the groups inhabiting the north-western and central part of the range by the second discriminant function. The scatter plot of scores of individual male specimens in the projection of the first two discriminant axes Fig. 2), showed that variability of group 7 – central Greece with Peloponnesus and Cyclades Islands – only slightly overlapped with the rest of the samples. In addition, variability of the sample from the southern and eastern part of the Balkans overlapped, but was considerably more prominent than that exhibited by the geographic groups from the central and western parts of the range. The geographic groups from the wide-ranging area, including central northern Italy, southern Austria, Slovenia, Croatia, Bosnia & Herzegovina, Montenegro, Serbia (except the southernmost part), central Albania, north-western Bulgaria, north-western Macedonia and south-western Romania (1–5) as well as from the easternmost part of the species range (eastern Turkey, Georgia and Armenia – 8) showed lack of clear discrimination. In general, the geographic groups from the southern and eastern parts of the Balkan peninsula exhibited a higher degree of morphological differentiation than the groups from the central and western part of the species range. The area where variability of the samples overlapped could denote the position of the specimens belonging to the transitional populations.

Post hoc tests of significant discriminative characters (Table 3) showed that the most prominent differences among re-defined subspecies were displayed by the following characters: number of ventral (V) and subcaudal scales (S) and height of rostral plate (L scr). Thus, *V. a. meridionalis* was characterised by the presence of significantly lower numbers of ventral and subcaudal plates as compared to all other subspecies. On the other hand, *V. a. montandoni* was characterized by the higher values of rostral plate height and head width comparing to all other re-defined taxa. The two above-mentioned taxa differed in the number of subcaudal and ventral plates as well as in the values of horn height (Table 2).

Qualitative Data

Correspondence analyses. Unexpectedly, analyses of qualitative traits showed a quite similar differentiation pattern of geographic groups (Fig. 5). When character VI (type of dorsal trunk pattern) was excluded from the analyses due to possible subjectivity of the coding, samples were differentiated into three groups defined by the specific frequencies of characters and states. The first group consists of the samples from the eastern (and partly southern) part of the Balkans (6) and was defined by the following characters and combination of states: I:0 (suprarostral plate lacking); II:0 (neither nasorostral plate in contact with canthus

TABLE 4. Classification of cases of re-defined subspecies.

	% of exact classification	*V. a. ammodytes*	*V. a. montandoni*	*V. a. meridionalis*	*V. a. transcaucasiana*
V. a. ammodytes	97.62	287	7	0	0
V. a. montandoni	88.31	9	68	0	0
V. a. meridionalis	87.50	1	2	21	0
V. a. transcaucasiana	20.00	3	1	0	1
Total	94.25	300	78	21	1

rostralis); IV:1 (greater height of rostral plate); V:1 (greater height of rostral than nasorostral plates) and VII: 4, 5 and 6 (types of head pattern). The second group consist of samples from the southernmost part of the Balkans (7) and was characterized by the specific frequencies of characters and states: I:3 (three suprarostral plates); II:0 (neither nasorostral plate in contact with canthus rostralis); IV:0 (equal height and width of rostral plate); V:0 (heights of rostral and nasorostral plates equal) and VIII:0 (lower lip spot undivided). The third group contains all of the samples from the central and the western part of the range (1–5) and is defined by a numerous specific characters and states. The geographic group from the easternmost part of the range (8) was characterized by the mixture of state frequencies defining the above-mentioned three groups. Geographic groups from the southern and the eastern part of the Balkans displayed a considerably higher degree of differentiation upon analyses of qualitative traits.

DISCUSSION

Redefined Subspecies and Distribution Ranges

As confirmed by a previous study (Tomovic & Dzukic, 2003), only a combination of morphometric, meristic and qualitative characters could be taxonomically informative in analyses of morphological differentiation and systematics of nose-horned vipers.

Results of both discriminant and correspondence analyses showed a lack of clear morphological differentiation among the samples belonging to the geographic groups conventionally classified as *Vipera ammodytes ammodytes*, *V. a. gregorwallneri*, *V. a. ruffoi* and *V. a. illyrica*. Variability of all analysed morphological characters corresponded to the description of the nominate subspecies, and hence the former taxa should be regarded as synonyms of *V. a. ammodytes*, at least at the morphological level. Thus, the distribution of *V. a. ammodytes* would include central northern Italy, southern Austria, Slovenia, Croatia, Bosnia & Herzegovina, Montenegro, Serbia (excluding the southernmost part), northern Albania and north-western Macedonia as well as north-western Bulgaria and western Romania (Fig. 6). Contrary to the situation in the central and western parts of the range, morphological differentiation of geographic groups from the eastern and southern parts of the range was more complicated. Results of discriminant and correspondence analyses showed a considerably higher degree of differentiation of geographic groups from the southern and the eastern parts of the Balkans.

The highest degree of differentiation was displayed by the geographic groups from the southernmost part of the Balkan peninsula – central Greece, Peloponnesus and Cyclades. Since morphological characteristics corresponded to the subspecies *V. a. meridionalis*, I suppose that the "typical" populations of this subspecies most probably inhabit only the areas mentioned (Fig. 6).

Our previous studies revealed lack of clear morphological differentiation among the samples from the northern and the southern parts of Bulgaria, FYR of Macedonia and the southernmost part of Serbia (Tomovic & Dzukic, 2003). Morphological analyses and hybridization experiments with the samples from the Bulgarian part of the range also rejected the validity of subspecies *V. a. montandoni* (Christov *et al.*, 1997; Christov & Beshkov, 1999; Beshkov & Nanev, 2002) and treated it as a synonym of *V. a. meridionalis*. Morphological analyses of the specimens from Istanbul, Adapazari, Bursa and Kusadasi demonstrated (Tok & Kumlutas, 1996; Kutrup, 1999) that the European and the western part of Asiatic Turkey could be inhabited either by *V. a. meridionalis* or by *V. a. montandoni*. Golay *et al.* (1993) included *V. a. montandoni* in the list of synonyms of *V. a. meridionalis*. In addition, recent phylogenetic analyses of two samples from Bulgaria (assigned as belonging to *V. a. meridionalis* and *V. a. montandoni*) revealed that their distance divergence was very small – 0.4% (Garrigues *et al.*, 2005). Based upon this, they also suggested that *V. a. montandoni* should be synonymous with *V. a. meridionalis*. However, in the experimental studies of Christov & Beshkov (1999), Beshkov & Nanev (2002) and Garrigues *et al.* (2005), different populations of *V. a. meridionalis* (from Greece and/or western Turkey) were not compared, and they assumed southern Bulgarian populations to be *V. a. meridionalis*.

In contrast, this study clearly shows that the geographic groups from the eastern and partly southern parts of the Balkans were rather different from those inhabiting the southernmost part of the Balkan peninsula, and hence, might be classified as *V. a. montandoni*. Thus, the range of this subspecies might include: SE Romania, most of Bulgaria (excluding NW part), western (European) Turkey, NE, N, NW Greece, FYR Macedonia (excluding NW part), the southernmost part of Serbia and southern and central Albania (Fig. 6). It should be noted that populations of the nose-horned viper inhabiting these areas (with exception of SE Romania and NE Bulgaria) were previously treated as populations belonging to *V. a. meridionalis* (Buresch & Zonkow, 1934; Karaman, 1939; Radovanovic, 1951; Biella, 1983; Dzukic, 1995; Crnobrnja-Isailovic & Haxhiu, 1997; Beshkov & Nanev, 2002; Vozenilek, 2002).

The geographic groups from the eastern part of Turkey, Georgia and Armenia were generally treated as subspecies *V. a. transcaucasiana* (Eiselt & Baran, 1970; Tok & Kumlutas, 1996; Kutrup, 1999 and references therein). On the other hand, this subspecies has been raised to specific level by some authors (Obst, 1983; Nilson *et al.*, 1999; Herrmann *et al.*, 1987). Although this taxon was originally described on the basis of a few qualitative traits only, and one of the "diagnostic" characters was the broken zig-zag band (e.g. "dorsal zig-zag band... is replaced by a series of nar-

row, transverse bars... which, on some parts of the body, break up into pairs forming two alternating series...", see Boulenger, 1913), the dorsal trunk pattern was not included in my analysis due to possible subjectivity of the scoring (see Tomovic & Dzukic, 2003). Additionally, during inspection of all 922 specimens from different (mostly southern and eastern Balkans) areas, I found specimens having both partly (39) or completely (nine) broken dorsal bands (not presented here). The presence of a specific type of dorsal band may have resulted from "ecogenesis, i.e. different current selection pressures leading to adaptive modifications of morphological characters" (Wüster *et al.*, 1992). Since the results of multivariate analyses of both quantitative and qualitative traits showed that there was no specific morphological differentiation of the geographic groups from the easternmost part of the range, this could imply that there is no justification for raising *V. a. transcaucasiana* to the species level based on apparent differences in a single character and/or distribution gap. These findings are consistent with the results of the phylogenetic analyses of Garrigues *et al.* (2005), who showed that the sample of *V. a. transcaucasiana* was less distant than *V. a. ammodytes*, and that the "*V. ammodytes* complex" represented a monophyletic group.

Transitional Zones

Since these analyses made it possible to re-define the subspecies and propose new geographic ranges, transitional zones had to be modified as well. Specimens of re-defined subspecies having canonical scores within overlapping zones (Fig. 3) and that were misclassified (Table 4) could belong to transitional populations. Intergradation zones between *V. a. ammodytes* and *V. a. montandoni* would include western Bulgaria, eastern, south-eastern and southern Serbia, the north-western part of FYR of Macedonia and central Albania (Fig. 6). These zones were quite precisely defined, but pertained to *V. a. ammodytes* and *V. a. meridionalis* (Buresch & Zonkow, 1934, see figure 38; Karaman, 1939). The northern continental part of Greece and probably the southernmost part of Albania might represent intergradation zones between *V. a. meridionalis* and *V. a. montandoni* (Fig. 6). Specimens from the Bosporus region could belong to transitional populations between *V. a. montandoni* and *V. a. transcaucasiana*.

NW to SE Gradient of Degree of Differentiation

The most intriguing result of this study is the pronounced increasing north-west to south-east gradient of morphological differentiation. It was demonstrated that a similar pattern of increasing gradient of morphological degree of differentiation NW to SE was displayed by populations of the Eastern grass snake (*Natrix natrix natrix*) and populations from Greece and Turkey were assigned as "those near the root of the eastern phylogenetic tree" (Thorpe, 1984). Recently, a lot of integrative studies of molecular phylogeography have shown that animal and plant taxa from southern areas of Europe display significantly higher degrees of genetic differentiation and diversity than those from central and northerns part of Europe (e. g. Hewitt, 1996, 1999; Taberlet *et al.*, 1998 and references therein). These "large-scale" studies designated the Iberian, Apennine and Balkan peninsulas as the main refugia of the European biota during the Pleistocene glaciations. Some hypotheses suggest that "a reduction in diversity from southern to northern Europe in the degree of allelic variation and species subdivision... is attributed to rapid expansion northward and the varied topography of southern refugia allowing populations to diverge through several ice ages" (Hewitt, 1996, 1999). As was shown for the grasshopper (see Hewitt, 1999), populations of the Balkans (probably western or central parts) whose northern populations formed the leading edge of postglacial expansion, display less haplotype diversity than those from Greece and Turkey. To my knowledge, "medium-scale" studies that analysed genetic (or morphological) patterns of diversity of amphibian and/or reptile species from one of the refugia exclusively (especially the Balkans), have rarely been conducted (e.g. *Rana latastei* from Italy and Slovenia – east-to-west gradient of genetic diversity shown by Garner *et al.*, 2004; Iberian Peninsula – Gómez & Lunt, 2004, and references therein).

CONCLUSIONS

Phenotypic variation could sometimes reflect genotypic variation, but could also be simply the result of environmental variation, or both. Despite the fact that these analyses provide evidence for the validity of four subspecies, some may argue that they should not be recognized as separate taxa, as morphological differentiation of geographic groups does not appear to be categorical, but clinal (Fig. 4). Without phylogeographic analysis, we can only assume that the aforementioned "large-scale" pattern of genetic and morphological diversity could be introduced into "medium-scale" genetic and/or morphological differentiation schemes for the nose-horned viper. In any case, for a systematic overview of this species, further phylogenetic studies are required.

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REFERENCES

Arnold, E. & Ovenden, D. (2002). *A Field Guide to the Reptiles and Amphibians of Britain and Europe*, 2nd edition. London: Collins.

Beshkov, V. & Nanev, K. (2002). *Zemnovodni i vlecugi v Blgaria* (*Amphibians and Reptiles in Bulgaria*). Sofia–Moskva: Pensoft.

Biella, H. (1983). *Die Sandotter*. Wittenberg Lutherstadt: A. Ziemsen Verlag.

Biella, H. & Blättler, E. (1989). Problematik der unterartlichen Eingliederung nord- und südwestlicher *Vipera ammodytes* – Populationen, dargestellt am Beispiel der südslowenischen Sandviper (Reptilia, Serpentes: Viperidae). *Zoologische Abhandlungen Staatliches Museum Fur Tierkunde in Dresden* **45**, 11-22.

Boulenger, G. A. (1913). On the geographical races of *V. ammodytes*. *Annals and Magazine of Natural History* **11**, 283-287.

Brodmann, P. (1987). *Die Giftschlangen Europas und die Gatting Vipera in Afrika und Asien*. Bern: Kümmerly-Frey.

Buresch, I. & Zonkow, J. (1934). Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkanhalbinsel, II – Schlangen (Serpentes). *Mitteilungen aus dem Naturwissenschaftlicher Institut in Sofia* **7**, 106-188.

Christov, K. V. & Beshkov, V. A. (1999). On the subspecies morphological characteristics of the Sand Vipers (*Vipera ammodytes*) from different locations in Bulgaria. *Acta Zoologica Bulgarica* **51**, 61-68.

Christov, K., Matev, I. & Iliev, Y. (1997). Major characteristics of three *Vipera ammodytes* subspecies from laboratory conditions. *Folia Zoologica* **46**, 286-288.

Crnobrnja-Isailovic, J. & Haxhiu, I. (1997). *Vipera ammodytes* (Linnaeus, 1758). In: *Atlas of Amphibians and Reptiles in Europe*, 384-385. Gasc, J.-P., Cabela, A., Crnobrnja-Isailovic, J., Dolmen, D., Grossenbacher, K., Haffner, P., Lescure, J., Martens, H., Martinez Rica, J. P., Maurin, H., Oliveira, M. E., Sofianidou, T. S., Veith, M. & Zuiderwijk, A. (Eds). Paris: Societas Europaea Herpetologica and Muséum National d'Histoire Naturelle.

Dzukic, G. (1995). Diverzitet vodozemaca (Amphibia) i gmizavaca (Reptilia) Jugoslavije, sa pregledom vrsta od meunarodnog znacaja. In: *Biodiverzitet Jugoslavije*, 449-469. Stevanovic, V. & Vasic, V. (Eds). Beograd: Biološki fakultet & Ecolibri.

Eiselt, J. & Baran, I. (1970). Ergebnisse zoologischer Sammelreisen in der Türkei: Viperidae. *Annalen des Naturhistorischen Museum Wien* **74**, 357-369.

Fuhn, I. & Vancea, S. (1961). *Fauna Republicii Populare Romine. Reptilia. XIV/2*. Bucharest: Academia Republicii Populare Romane.

Garner, T. W. J., Pearman, P. B. & Angelone, S. (2004). Genetic diversity across a vertebrate species' range: a test of the central-peripheral hypothesis. *Molecular Ecology* **13**, 1047–1053.

Garrigues, T., Dauga, C., Ferquel, E., Choumet, V. & Failloux, A.-B. (2005). Molecular phylogeny of *Vipera* Laurenti, 1768 and the related genera *Macrovipera* (Reuss, 1927) and *Daboia* (Gray, 1842), with comments about neurotoxic *Vipera aspis aspis* populations. *Molecular Phylogenetics and Evolution* **35**, 35–47.

Golay, P., Smith, H. M., Broadley, D. G., Dixon, J. R., McCarthy, C., Rage, J.-C., Schätti B. & Toriba, M. (1993). *Endoglyphs and Other Major Venomous Snakes of the World. A Checklist*. Geneva: Azemiops.

Gómez, A. & Lunt, D. H. (2004). Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula. In: *Phylogeography in Southern European Refugia: Evolutionary Perspectives on the Origins and Conservation of European Biodiversity*. Weiss, S. & Ferrand, N. (Eds.). Dordrecht: Kluwer Academic Publishers, in press.

Herrmann, H.-W., Joger, U., Nilson, G. & Sibley, C. G. (1987). First steps towards a biochemically based reconstruction of the phylogeny of the genus *Vipera*. In: *Proceedings of the 4th Ordinary General Meeting of Societas Europaea Herpetologica*, 195-200. Van Gelder, J. J., Strijbosch, H. & Bergers, P. J. M. (Eds.). Nijmegen: Faculty of Sciences.

Hewitt, G. M. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**, 247-276.

Hewitt, G. M. (1999). Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* **68**, 87-112.

Karaman, S. (1939). Über die Verbreitung der Reptilien in Jugoslavien. *Annales Musei Serbiae Meridionalis* **1**, 1-20.

Kutrup, B. (1999). The morphology of *Vipera ammodytes transcaucasiana* (Reptilia, Viperidae) specimens collected from Murgul (Artvin, Turkey). *Turkish Journal of Zoology* **23**, 433-438.

Manly, F. J. B. (1986). *Multivariate Statistical Methods: A Primer*. New York: Chapman and Hall.

McDiarmid, R. W., Campbell, J. A. & Touré, T. (1999). *Snake Species of the World. A Taxonomic and Geographic Reference*. Washington D.C.: The Herpetologists' League.

Nilson, G., Tuniyev, B., Andrén, C., & Orlov, N. (1999). Vipers of Caucasus: taxonomic considerations. *Kaupia* **8**, 103-106.

Obst, F. J. (1983). Zur Kenntnis der Schlangengattung *Vipera* (Reptilia, Serpentes, Viperidae). *Zoologische Abhandlungen Staatliches Museum Fur Tierkunde in Dresden* **38**, 229-235.

Radovanovic, M. (1951). *Vodozemci i gmizavci naše zemlje. (Amphibien und Reptilien Jugoslawiens).* Beograd: Nauèna knjiga.

Rohlf, F. J. (1988). *NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System.* New York: Exeter Publishing.

Sochurek, E. (1972). Zur Verbreitung und Systematik europäischer Giftschlangen. *Vivarium* **2**, 33-36.

Sochurek, E. (1976). Zur systematischen Stellung der alpinen Hornotter. *Carinthia II* **166/86**, 447-452.

Sochurek, E. (1985). Gedanken zur Hornottern-Systematik. *Elaphe* **2**, 23-26.

StatSoft, Inc. (1997). *STATISTICA release 5 (Quick Reference)*, 3rd Edition. Tulsa.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.-G. & Cosson, J.-F. (1998). Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* **7**, 453-464.

Thorpe, R. S. (1984). Multivariate patterns of geographic variation between the island and mainland populations of the Eastern grass snake (*Natrix natrix natrix*). *Journal of Zoology* **204**, 551-561.

Thorpe, R. S. (1987). Geographic variation: A synthesis of cause, data, pattern and congruence in relation to subspecies, multivariate analysis and phylogenesis. *Bolletino di Zoologia* **54**, 3-11.

Tok, C. V. & Kumlutas, Y. (1996). On specimens of *Vipera ammodytes transcaucasiana* (Viperidae) from Persembe, Black Sea region of Turkey. *Zoology in the Middle East* **13,** 47-50.

Tomovic, L. J. & Dzukic, G. (2003). Geographic variability and taxonomy of the nose-horned viper, *Vipera ammodytes* (L. 1758), in the central and eastern parts of the Balkans: a multivariate study. *Amphibia-Reptilia* **24,** 359-377.

Tomovic, L. J., Radojicic, J., Dzukic, G. and Kalezic, L. M. (2002). Sexual dimorphism of the Sand Viper (Vipera ammodytes L.) from the central part of Balkan peninsula. *Russian Journal of Herpetology* **9**, 69-76.

Ulber, T. (Ed.) (1994). *Catalogue of Valid Species and Synonyms. Volume 2. Viperinae and Atractaspididae.* Bredell, S.A.: Herprint International.

Vozenilek, P. (1971). Zmije ruzkata – *Vipera ammodytes* (L.). *Ziva Praha* **1**, 28-29.

Vozenilek, P. (2002). Stále obdivovaná zmije ruzkatá *Vipera ammodytes* (Linnaeus, 1758). *Akvárium Terárium* **45**, 70-75.

Vozenilek, P. & Cizek, P. (1978). New aspect for determination of Balkan subspecies of *Vipera ammodytes* (Linnaeus, 1758). *Fauna Bohemiae Septentrionalis* **3**, 69-81.

Wüster, W. & Broadley, D. G. (2003). A new species of spitting cobra (*Naja*) from north-eastern Africa (Serpentes: Elapidae). *Journal of Zoology* **259**, 345-359.

Wüster, W. & Thorpe, R. S. (1987). Geographic variation in the cobras of the genus *Naja* in southeast Asia: a multivariate analysis. In: *Proceedings of the 4th Ordinary General Meeting of Societas Europea Herpetologica*, 449-452. Van Gelder, J. J., Strijbosch, H. & Bergers, P. J. M. (Eds.) Nijmegen: Faculty of Sciences.

Wüster, W., Otsuka, S., Thorpe, R. S. & Malhotra, A. (1992). Morphological variation in Russell's viper in Burma and Thailand. *Herpetological Journal* **2**, 99-101.

Wüster, W., Thorpe, R. S., Cox, M. J. Jintakune, P. & Nabhitabhata, J. (1995). Population systematics of the snake genus *Naja* (Reptilia: Serpentes: Elapidae) in Indochina: multivariate morphometrics and comparative mitochondrial DNA sequencing (cytochrome oxidase I). *Journal of Evolutionary Biology* **8**, 493-510.

Zuffi, M. A. L. (2002). A critique of the systematic position of the asp viper subspecies *Vipera aspis aspis* (Linncus, 1758), *Vipera aspis atra* Meisner, 1820, *Vipera aspis francisciredi* Laurenti, 1768, *Vipera aspis hugyi* Schinz, 1833 and *Vipera aspis zinnikeri* Kramer, 1958. *Amphibia-Reptilia* **23**, 191-213.

Zuffi, M. A. L. & Bonnet, X. (1999). Italian subspecies of the asp viper, *Vipera aspis*: patterns of variability and distribution. *Italian Journal of Zoology* **66**, 87-95.

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THE TADPOLE OF *PHYSALAEMUS FERNANDEZAE* (ANURA: LEPTODACTYLIDAE)

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This paper describes the external and buccopharyngeal morphology, chondrocranium and cranial muscles in tadpoles of *Physalaemus fernandezae*. The data are compared with those for other species of *Physalaemus* to improve the diagnosis of the "species group" within the genus. Species of the "*P. biligonigerus*" group have four infralabial papillae, two semicircular arches of pustulations in a V-shaped pattern on the prenarial arena, 6–8 conical papillae and 40–60 pustulations on the buccal roof arena, four postnarial papillae, a semicircular median ridge, claw-shaped lateral ridges and larval crista parotica with a poorly-developed anterior process. Species of the "*P. pustulosus*" group possess four infralabial papillae (shared with the *P. biligonigerus* group), tooth row formula 2(2)/3, four lingual papillae, two postnarial papillae, twelve conical papillae and 16–20 pustulations on the buccal roof arena, short lateral ridges with rough concave margins and larval crista parotica with a well-developed anterior process and reduced posterior process. Species of the "*P. cuvieri*" group present two infralabial papillae, three pustulations and two serrated papillae on the prenarial arena, five pustulations and two serrated papillae on the postnarial arena, four long and bifid papillae and more than 60 pustulations on the buccal roof arena, and lack larval crista parotica. In species of the "*P. signiferus*" group both medial and lateral mental gaps are absent, and the tooth row formula is 2(2)/3(1).

Key words: amphibian, larvae, frog, morphology, musculature

INTRODUCTION

The neotropical genus *Physalaemus* comprises a group of small toad-like leptodactylid frogs distributed from Mexico to northern Argentina (Frost, 2004). Following Lynch (1970), four species groups of *Physalaemus* are currently recognized: the *P. cuvieri, P. biligonigerus*, *P. pustulosus* and *P. signiferus* groups.

At present, anuran tadpole morphology is receiving increasing attention in phylogenetic analyses (Larson & de Sá, 1998; Faivovich, 2002; Haas, 2003). Of the 48 species of *Physalaemus* (Caramaschi *et al.*, 2003; Cruz & Pimenta, 2004; Frost, 2004; Haddad & Sazima, 2004; Ron *et al.*, 2004, 2005), the tadpoles of only 20 have been described (Nomura *et al.*, 2003; Pimenta *et al.*, 2005). The buccopharyngeal morphology, chondrocranium and cranial muscles of *Physalaemus* larvae remain poorly known (Larson & de Sá, 1998; Palavecino, 2000; Nomura *et al.*, 2003).

Physalaemus fernandezae belongs to the "*P. cuvieri*" group and inhabits flooded grasslands in northeastern Argentina and southwestern Uruguay (Langone, 1994). Several studies have been carried out concerning the mating call, natural history and adult morphology of this species (Gallardo, 1963; Barrio, 1964, 1965; Lobo, 1992) but a detailed description of its tadpole is not available. Gallardo (1963), Barrio (1964), Cei (1980) and Langone (1994) give some information about total length and general aspects of the oral disc.

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The aim of this paper is to describe the external and buccopharyngeal morphology, chondrocranium and cranial muscles of *Physalaemus fernandezae* tadpoles in the context of the other *Physalaemus* species. This information will be used to improve the diagnosis of the *Physalaemus* species group, which so far has been based only on adult characters.

MATERIALS AND METHODS

Between May and July 2001, we collected tadpoles of *Physalaemus fernandezae* at Punta Lara (Buenos Aires province, Argentina). Some of them (n=13) were fixed after capture in 10% buffered formalin and then staged using Gosner's (1960) table. The material examined is deposited in the amphibian collection of the Museo de La Plata (MLP). The remaining tadpoles were reared until metamorphosis to corroborate the species identification. Seven tadpoles were employed for oral disc and external morphology descriptions (stages 32, 35, 36, 37 and 38, MLP 3333). Two stage 35 (MLP 3334) and three stage 40 (MLP 3335) specimens were stained following the technique of Taylor & Van Dyke (1985). The process was interrupted before clearing; tadpoles were dissected for observation of muscles and then cleared for chondrocranium description. One tadpole (stage 39) was dehydrated in a graded ethanol series (30%: three 15-minute baths; 50%: a week; 70%: three 15-minute baths; 100%: 15 minutes prior to the critical point) for scanning electronic microscope examination of the buccopharyngeal morphology and keratinized structures of the oral disc. The tadpole was sectioned according to Wassersug (1980) and critical point dried

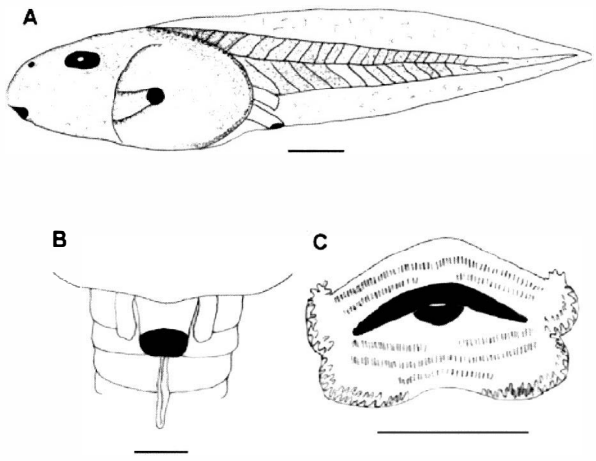

FIG. 1. External morphology of the tadpole of *Physalaemus fernandezae* at stage 38 (MLPA 3333). A) Lateral view; B) ventral view of the vent tube; C) oral disc. Scale bars=1 mm.

in carbon dioxide using amyl acetate as intermediate liquid, mounted on a double-face Carbon tape and sputter-coated with 400 Å thick gold-palladium using a Model Ion Sputter Fine Coat JFC-1100 (Jeol System). Photographs were taken using a Jsm-T100 scanning electron microscope at 5-15 kV equipped with an Ilford camera. The buccopharyngeal morphology of a stage 35 tadpole was also examined under a stereomicroscope. Observations, measurements and drawings referring to external morphology, chondrocranium and cranial muscles were made under a Reichert Wien stereomicroscope with measuring equipment (accurate to the nearest 0.1 mm) and camera lucida.

Terminology follows D'Heursel & de Sá (1999) and Haas (1995) for chondrocranium structures, Alcalde & Rosset (2003) for chondrocranial measurements, Haas (2001) for mandibular musculature, Haas & Richards (1998) and Haas (2003) for branchial and hyoid musculature, Schlosser & Roth (1995) for muscular innervation, Wassersug (1980) for buccopharyngeal morphology, Van Dijk (1966) and Lavilla (1983) for external morphology, Johnston & Altig (1986) for oral disc morphology and Altig & Johnston (1989) for tadpole ecomorphological types.

RESULTS

External Morphology

The following description is based on seven specimens at developmental stages 32–38. External morphology is illustrated in Fig. 1. Measurements are in mm (arithmetic mean ± 95% confidence limits). Percentages were calculated based on the maximum and minimum values of each variable.

Type IV, exotrophic, lentic and benthic tadpoles. Size small, total length 26.84 mm (±1.92), body length (8.73±0.84) one-third of total length; body shape oval, body length 50–60% of body height (4.96±0.60), and body width (5.23±0.32) 80–100% of body height, without constrictions between head and trunk; snout rounded in dorsal and lateral profile; eyes relatively large, dorsolaterally placed; eye diameter (1.21±0.11) 27–28% of body width at eye level (4.34±0.42), and 87–93% of interorbital distance (1.39±0.10); interorbital distance 29–32% of body width at eye level; rostro-orbital distance 5.86±0.26. Nostrils subcircular, dorsal, elevated, closer to tip of snout than to eye, nostril diameter (2.76±0.4) 13–14% of body width at nostril level, rostronasal distance (0.71±0.11) 55–61% of orbitonasal distance (1.06±0.14); nostril diameter (0.37±0.06) 37–45% of internarial distance (0.94±0.10), and internarial distance 66–73% of interorbital distance; extranarial distance (1.55±0.10) 45–47% of extraorbital distance (3.37±0.23). Spiracle sinistral, spiracular tube and opening lateral, spiracular opening rounded, rostro-spiracular distance

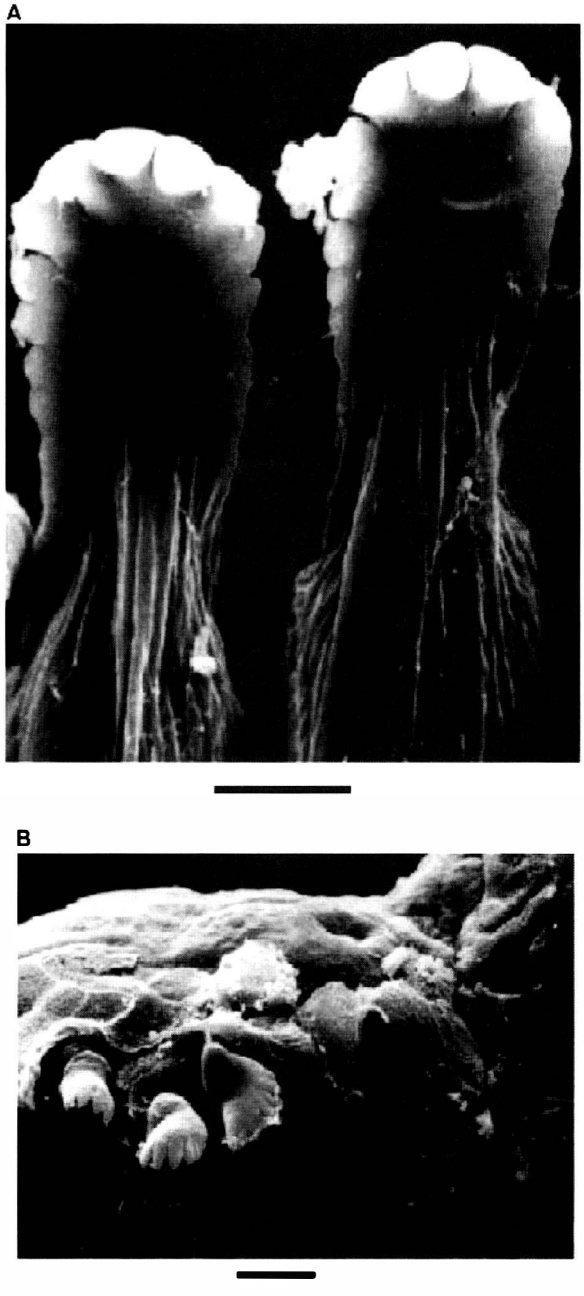

FIG. 2. Scanning electron microscope photographs of the keratodonts of the first mental row (A) and of the third left marginal papilla bearing small keratodonts (B) of *Physalaemus fernandezae* at stage 39. Scale bars 10 µm.

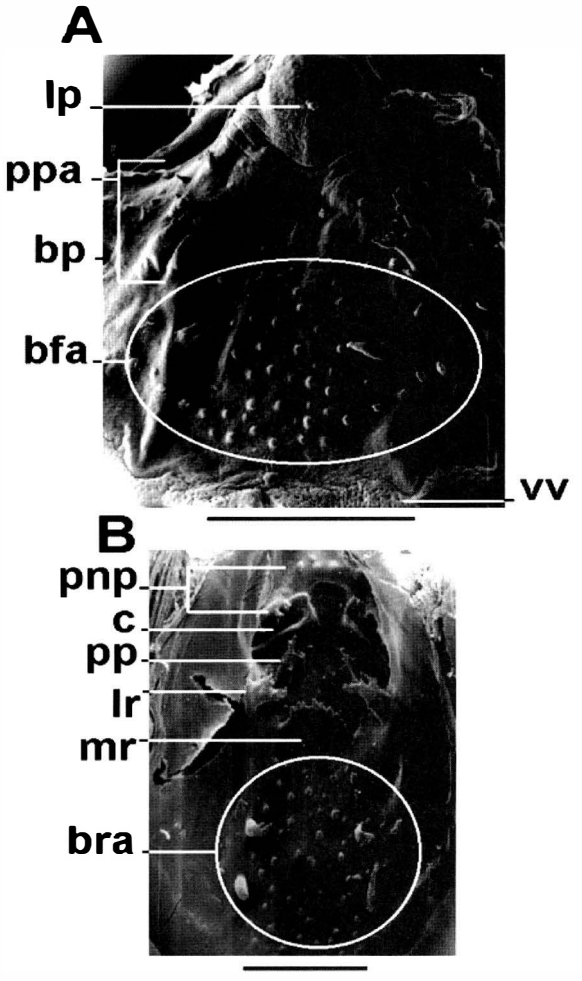

FIG. 3. Scanning electron microscope photographs of the buccal floor (A) and buccal roof (B) papillation of *Physalaemus fernandezae* at stage 39. In A, infralabial papillae are not visible. In B, infrarostral papillae are not visible. Scale bars 1 mm. References: bfa, buccal floor arena; bp, buccal pocket; bra, buccal roof arena; c, choana; lp, lingual papilla; lr, lateral ridge; mr, median ridge; pnp, prenarial papillae; pp, postnarial papillae; ppa, prepocket papillae; vv, ventral velum.

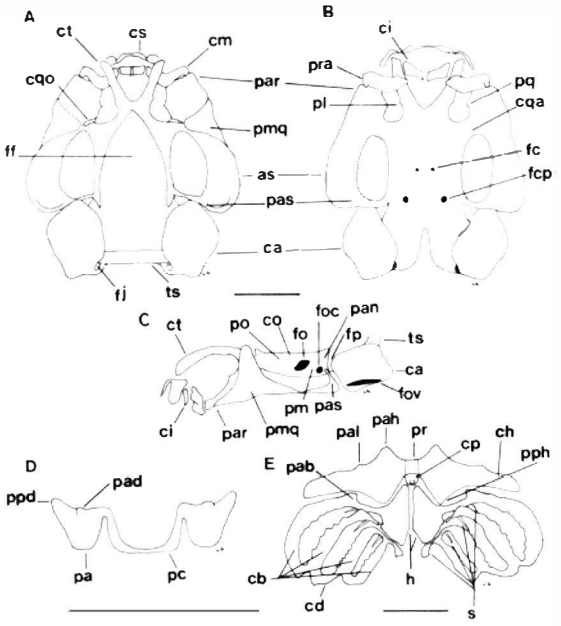

FIG. 4. Chondrocranium of *Physalaemus fernandezae* at stage 35 (MLPA 3334). A) Dorsal, B) ventral and C) lateral views of the neurocranium and mandibular arch. D) Frontal view of cartilago suprarostalis. E) Ventral view of hyobranchial apparatus. Dark areas represent cranial fenestrations. Scale bars 1 mm. References: as, arcus subocularis; ca, capsula auditiva; cb, ceratobranchiales; cd, commissura terminalis; ch, ceratohyale; ci, cartilago infrarostrale; cm, cartilago meckeli; co, cartilago orbitale; cp, copula posterior and processus urobranchialis; cqa, commissura quadrato-cranialis anterior; cqo, commissura quadrato-orbitalis; cs, cartilago suprarostralis; ct, cornu trabeculae; fc, foramen craneopalatinum; fcp, foramen caroticum primarium; ff, fenestra frontoparietalis; fj, foramen jugulare; fo, foramen opticum; foc, foramen oculomotorium; fov, fenestra ovalis; fp, fissura prootica; h, hypobranchiales; pa, pars alaris; pab, processus anterior branchialis; pad, processus anterior dorsalis; pah, processus anterior hyalis; pal, processus anterolateralis hyalis; pan, pila antotica; par, processus articularis; pas, processus ascendens; pc, pars corporis; pl, processus lateralis; pm, pila metoptica; pmq, processus muscularis quadrati; po, pila preoptica; ppd, processus posterior dorsalis; pph, processus posterior hyalis; pq, processus quadrato-ethmoidalis; pr, pars reuniens; pra, processus retroarticularis; s, spicula I, II III and IV; ts, tectum synoticum.

(5.86±0.26) 63–71% of body length. Vent tube length (2.08±0.75) 14–29% of body length; vent opening medial. Tail length (15.79±1.57) 59–63% of total length, tail height at the base of the tail 5.39±0.55, tail height at the tip of the caudal musculature 0.70±0.26; dorsal and ventral fins well developed, with slightly curved margins; maximum tail height approximately at middle length and lower than body height; tail axis straight and tip of tail rounded. Caudal musculature height at the base of the tail (2.74±0.36) 55–56% of body height, caudal musculature width at the base of the tail 2.51±0.27; myotomes clearly visible, the posteriormost ones not reaching the end of the tail.

Oral disc sub-terminal, not visible dorsally; oral disc width 1.91±0.14, disc small, about 36–38% of maximum body width; disc with angular constrictions; an irregular double row of triangular and rounded marginal papillae in lateral regions; small mental gap present (0.43±0.11); with medium-sized rostral gap (1.16±0.08), about 61% of oral disc width; intramarginal papillae absent; tooth row formula 2(2)/3(1), rostrodonts well developed and keratinized, margins serrated (Fig. 1C); keratodonts spatulated and serrated (Fig. 2A). One specimen bears small keratodonts on the marginal papillae (Fig. 2B).

In life, dorsum and lateral body sides uniformly greyish, darker dorsally than laterally; ventral region grey, peribranchial zone paler than abdominal region, abdomen rich in guanophores producing silvery and golden sheens; fins scantily pigmented, transparent, and dotted with few irregular rows of melanophores; caudal musculature darker with melanophores arranged more densely than on fins. In preservative, creamy white tail with few isolated brown spots more abundant in the hypaxial musculature. Body darker than tail, dorsally dark-brown, ventrally pale brown. Intestinal mass visible through transparency.

Buccopharyngeal morphology. The buccal floor (Fig. 3A) possesses two short and rounded infralabial papillae (not visible in Fig. 3A), and one triangular lingual papilla. The buccal floor arena has more than 60 pustulations and six large and serrated papillae (Fig. 3A). There are 12 short and conical papillae (sometimes serrated) and few pustulations on the prepocket arena. The ventral velum presents secretory pits.

The buccal roof (Fig. 3B) possesses two trifid infrarostral papillae (not visible in Fig. 3B). Prenarial arena with three pustulations and two long and trifid papillae. The postnarial arena presents three central pustulations and two lateral and serrated papillae placed anteriorly to the median ridge, and one small pustulation anterior to each lateral ridge. The well-developed median ridge is subcircular and serrated. The lateral ridges are rectangular and serrated. There are more than 60 central pustulations, and four long and bifid papillae on the buccal roof arena.

Chondrocranium. Neurocranium almost rectangular (width/length=0.86) and depressed (height/width=0.4), with greatest width at level of the arcus subocularis. Medial corpora of the cartilago suprarostralis connected by a distal bridge (Fig. 4D). Lateral partes alares and partes corpora joined by a proximal connection. Well-developed processus posterior dorsalis. Cornua trabeculae forming about 19% of chondrocranial length, uniformly wide, with well-developed processus lateralis. The cranium is roofed only between the capsula auditivae by the tectum synoticum. Lateral walls (cartilagines orbitales) formed by the pila ethmoidea (*sensu* de Beer, 1985), pila preoptica, pila metoptica and pila antotica (Fig. 4C). Basi cranii closed and pierced by the foramina carotica primaria and craneopalatina (Fig. 4B). Capsulae auditivae subspherical representing about 38% of the chondrocranial length; dorsally coupled with the processus ascendens and lacking larval crista parotica. Medial walls of the capsula auditivae pierced by the acoustic and the endolimphatic foramina. No inferior perilimphatic foramen at the studied stages. Superior perilimphatic foramen opening in the posterior wall of the capsula auditiva, just in front of the jugular foramen. Operculum not chondrified.

Palatoquadrate with processus articularis quadrati, processus muscularis quadrati, commissura quadrato-cranialis anterior, processus quadrato-ethmoidalis and processus ascendens, but lacking commissura quadrato-orbitalis, processus pseudopterygoideus and larval processus oticus. No lateral projections on the margins of the arcus subocularis, and processus ascendens joined to pila antotica by an intermediate union (Fig. 4C).

Lower jaw consisting of cartilago meckeli and cartilagines infrarostrales. Processus retroarticularis of cartilago meckeli short and articulating with processus articularis quadrati. Both processus ventromedialis and dorsomedialis of the cartilago meckeli articulating with

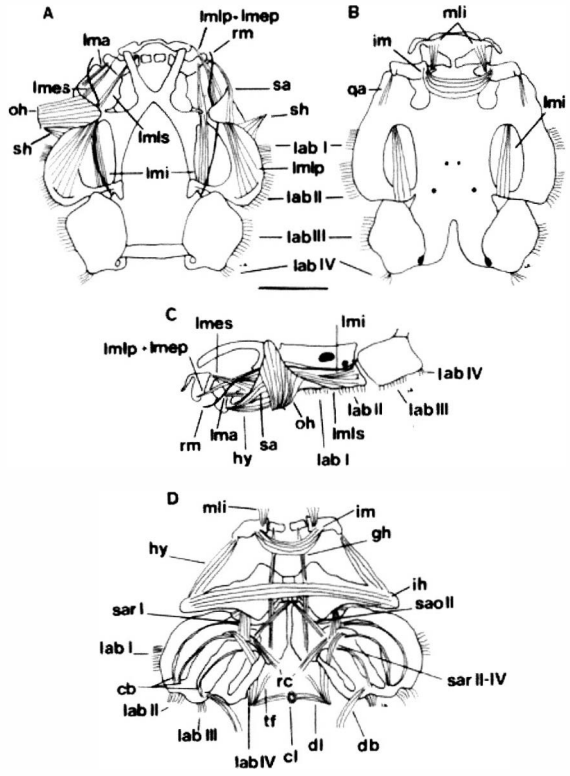

FIG. 5. Cranial muscles of *Physalaemus fernandezae* at stage 35 (MLPA 3834). A) Dorsal, B) ventral and C) lateral views of muscles related to neurocranium and mandibular arch. d) Ventral view of muscles related to the hyobranchial apparatus. In A, mm. levator mandibulae externus profundus, levator mandibulae longus profundus, suspensorioangularis (left side), levator mandibulae longus superficialis, levator mandibulae externus superficialis and orbitohyoideus (right side) were removed. Dark areas represent cranial fenestrations. Scale bar 1 mm. References: cb, constrictor branchiales II, III and IV; cl, constrictor laryngis; db, diaphragmatobranchialis; dl, dilatator laryngis; gh, geniohyoideus; hy, hyoangularis lateralis; ih, interhyoideus; im, intermandibularis; lab I, levator arcuum branchialium I; lab II, levator arcuum branchialium II; lab III, levator arcuum branchialium III; lab IV, levator arcuum branchialium IV; lma, levator mandibulae articularis; lmep, levator mandibulae externus profundus; lmes, levator mandibulae externus superficialis; lmi, levator mandibulae internus; lmlp, levator mandibulae longus profundus; lmls, levator mandibulae longus superficialis; mli, mandibulolabialis inferior; oh, orbitohyoideus; qa, quadratoangularis; rc, rectus cervicis; rm, ramus mandibularis of trigeminus nerve; sa, suspensorioangularis; sao II, subarcualis obliquus II; sar I, subarcualis rectus I; sar II–IV, subarcualis rectus II–IV; sh, suspensoriohyoideus; tf, tympanopharyngeus.

the cartilagines infrarostrales by the sindesmotic commissura intramandibularis (Fig. 4B).

Copula I absent. All ceratohyale processes are well developed, except the very short processus anterolateralis hyalis. Ceratohyalia medially joined by a rectangle-shaped pars reuniens. Copula II bearing a short processus urobranchialis. Ceratobranchiale I continuous with the hypobranchiale; the remaining ceratobranchiales sindesmotically joined to the hypobranchiale (Fig. 4E). Ceratobranchiales III and IV

TABLE 1. Origin and insertion of each mandibular and hyobranchial muscle on tadpoles of *Physalaemus fernandezae*.

Muscle	Origin	Insertion
NERVUS TRIGEMINUS (CRANIAL NERVE V), MANDIBULAR MUSCULATURE		
Levator mandibulae internus	Processus ascendens	Cartilago meckeli
Levator mandibulae longus superficialis	Arcus subocularis	Cartilago meckeli
Levator mandibulae longus profundus	Arcus subocularis	Both muscles insert
Levator mandibulae externus profundus	Processus muscularis quadrati	together in the pars alaris by a common tendon.
Levator mandibulae externus superficialis	Processus muscularis quadrati	Pars alaris
Levator mandibulae articularis	Processus muscularis quadrati	Cartilago meckeli
Levator mandibulae lateralis	Absent at the studied stages	
Submentalis	Absent at the studied stages	
Intermandibularis	Cartilago meckeli	Median raphe
Mandibulolabialis inferior	Cartilago meckeli	Oral disc
Mandibulolabialis superior	Absent	
NERVUS FACIALIS, (CRANIAL NERVE VII), HYOID MUSCULATURE		
Suspensoriohyoideus	Processus muscularis quadrati and arcus subocularis	Ceratohyale
Suspensorioangularis	Processus muscularis quadrati	Cartilago meckeli
Quadratoangularis	Anterior and ventral on the palatoquadrate	Cartilago meckeli
Hyoangularis lateralis	Ceratohyale	Cartilago meckeli
Hyoangularis medialis	Absent	
Interhyoideus	Ceratohyale	Median raphe
Interhyoideus posterior	These muscles were not found under	
Diaphragmatopraecordialis	dissections, but they may be observable in histological sections	
NERVUS GLOSSOPHARYNGEUS (CRANIAL NERVE IX), BRANCHIAL MUSCULATURE		
Levator arcuum branchialium I	Arcus subocularis	Commissura terminalis I
Subarcualis rectus I	The dorsal head on ceratobranchiale I	Ceratohyale
	The ventral heads on ceratobranchiales II and III	
Constrictor branchialis I	Absent	
NERVUS VAGUS (CRANIAL NERVE X), BRANCHIAL MUSCULATURE		
Constrictor branchialis II	Ceratobranchiale I	Commissura terminalis I
Constrictor branchialis III	Ceratobranchiale II	Commissura terminalis II
Constrictor branchialis IV	Ceratobranchiale III	Commissura terminalis III
Diaphragmatobranchialis	Peritoneal wall	Ceratobranchiale III
Levator arcuum branchialium II	Arcus subocularis	Commissura terminalis II
Levator arcuum branchialium III	Capsula auditiva	Commissura terminalis III
Levator arcuum branchialium IV	Capsula auditiva	Ceratobranchiale IV
Subarcualis obliquus II	Between ceratobranchiales II and III	Processus urobranchialis
Subarcualis rectus II-IV	Ceratobranchiale IV	Ceratobranchiale II
Tympanopharyngeus	M. levator arcuum branchialum IV	Pericardium
Dilatator laryngis	Capsula auditiva	Larynx
Constrictor laryngis	Forms an annulus rounding the larynx	
Transversus ventralis IV	Absent	
NERVUS HYPOGLOSSUS (SPINAL NERVE II), HIPOBRANCHIAL MUSCULATURE		
Geniohyoideus	Hypobranchiale	Cartilago infarostrale
Rectus cervicis	Peritoneal wall	Ceratobranchiales IIand III

joined by a commissura proximalis. Processus branchiales not closed. All spiculae well developed.

Ossifications. The parasphenoid is the only bone present at the studied stages.

Cranial muscles. The cranial muscle pattern of *Physalaemus fernandezae*'s tadpoles is shown in Figure 5. Table 1 provides details about the origin and insertion of each muscle. The ramus mandibularis of the nervus trigeminus runs laterally to all muscles levatorae mandibulae.

DISCUSSION

External tadpole morphology has been described for four species of the "*Physalaemus biligonigerus*" group: *Physalaemus biligonigerus* (Férnandez & Férnandez, 1921; Cei, 1980), *P. fuscomaculatus* (Nomura *et al.*, 2003), *P. nattereri* (Vizzoto, 1967; Cei, 1980) and *P. santafecinus* (Perotti & Céspedez, 1999); nine of the "*P. cuvieri*" group: *P. aguirrei* (Pimenta & Cruz, 2004), *P. albonotatus* (Kehr *et al.*, 2004), *P. centralis* (Rossa-Feres & Jim, 1993), *P. cuqui* (Perotti, 1997), *P. cuvieri* (Bokermann, 1962; Cei, 1980; Heyer *et al.*, 1990), *P. enesefae* (Duellman, 1997), *P. gracilis* (Langone, 1989), *P. henselii* (Barrio, 1964; Cei, 1980) and *P. riograndensis* (Prigioni & Garcia, 2001); three of the "*P. pustulosus*" group: *P. coloradorum* (Cannatella & Duellman, 1984), *P. petersi* (Duellman, 1978) and *P. pustulosus* (Breder, 1946); and five of the "*P. signiferus*" group: *P. atlanticus* (Haddad & Sazima, 2004), *P. bokermanni* (Cardoso & Haddad, 1985), *P. camacan* (Pimenta *et al.*, 2005), *P. maculiventris* (Bokermann, 1963) and *P. spiniger* (Haddad & Pombal, 1998). The tadpole of *Physalaemus rupestris* is also known but does not belong to any of the four species groups (Nascimento *et al.*, 2001).

We compared these tadpoles' descriptions with the tadpole of *Physalaemus fernandezae* in order to obtain a characterization of the known larvae of *Physalaemus*. In light of the present knowledge, *Physalaemus* larvae are small (total length=14.8–31.5 mm), possess medium-sized tail (43–68% of total length), ovoid body, rounded snout, dorsolateral eyes, dorsal fin higher than ventral fin and sub-terminal emarginated oral disc with rostral gap.

Some features of the larvae of *Physalaemus*, such as the vent tube opening, the mental gap, the marginal papillae row and the tooth row formula, are highly variable and do not exhibit unique states for each of the species groups proposed by Lynch (1970). The vent tube of most larvae is positioned medially, but its opening may be medial (*P. atlanticus*, *P. bokermanni, P. camacan*, *P. fernandezae*, *P. rupestris*, *P. spiniger*) or dextral (*P. albonotatus*, *P. cuqui*, *P. fuscomaculatus*, *P. maculiventris*, *P. nattereri*). The vent opening of *P. centralis* may be medial, dextral or sinistral within the same population. Previous authors have not made a clear difference between the position of the vent tube and the vent opening for other species. In them, the vent tube position (or the vent opening?) should be dextral (*P. biligonigerus*, *P. cuvieri*, *P. gracilis, P. riograndensis*, *P. santafecinus*) or sinistral (*P. enesefae*).

The marginal papillae is present as a single row in tadpoles of most species of *Physalaemus* (*P. albonotatus*, *P. biligonigerus, P. bokermanni, P. centralis, P. cuqui, P. cuvieri, P. fuscomaculatus, P. maculiventris, P. nattereri, P. petersi, P. pustulosus, P. riograndensis, P. rupestris*). In other species, the marginal papillae row may be ventrally double and laterally single (*P. atlanticus, P. spiniger*), completely double (*P. gracilis*), ventrally single and double at some areas of the lateral region (*P. fernandezae*), ventrally single and double at the infra-angular areas (the internal rows of *P. fuscomaculatus* and *P. santafecinus* were described as intramarginal papillae by Perotti & Céspedez, 1999, and Nomura *et al.*, 2003), ventrally and laterally single but double or triple at the lateral folds (*P. coloradorum*), or laterally single but double or triple at mental region (*P. camacan*).

In anuran tadpoles, the marginal papillae row may be incomplete for lacking either the anterior (rostral gap) or posterior papillae (mental gap – here identified as medial mental gap). Some species of *Physalaemus* have two ventrolateral gaps on each side of the oral disc, here identified as lateral mental gaps. According to these types of mental gaps, larvae of *Physalaemus* may possess four oral disc configurations: (1) medial mental gap present and lateral mental gaps absent (*P. fernandezae*, *P. henselii)*; (2) both medial and lateral mental gaps present (*P. albonotatus, P. cuqui)*; (3) both mental gaps absent (*P. atlanticus, P. biligonigerus, P. bokermanni, P. camacan, P. coloradorum, P. enesefae, P. gracilis, P. maculiventris, P. nattereri, P. petersi, P. pustulosus, P. riograndensis, P. rupestris, P. santafecinus, P. spiniger*); and (4) only lateral mental gaps present (*P. centralis*). *Physalaemus fuscomaculatus* is unique in having configurations 3 and 4 within a single population. Contradictory information has been published about this character state for *P. cuvieri.* This species was described as possessing configurations 1 (Bokermann, 1962) and 4 (Heyer *et al.*, 1990).

There are seven tooth row formulae in *Physalaemus*: 2(2)/3(1) (*P. albonotatus, P. atlanticus, P. bokermanni, P. camacan, P. cuqui, P. fernandezae, P. gracilis, P. maculiventris, P. nattereri, P. spiniger*); 2(2)/2 (*P. biligonigerus, P.centralis*); 2(2)/2(1) (*P. fuscomaculatus, P. riograndensis, P. santafecinus*); 2/3(1) (*P. cuvieri, P. henselii*); 2(1)/3 (*P. enesefae*); 2(2)/3 (*P. coloradorum, P. petersi, P. pustulosus)*, or 2(2)/3(1-2) (*P. rupestris*). The tooth row formula 2(2)/3 is unique to the species assembled in the "*P. pustulosus*" species group.

The buccopharyngeal papillation has been described for *Physalaemus biligonigerus*, *P. fuscomaculatus*, *P. nattereri*, and *P. santafecinus* ("*P. biligonigerus*" group), *P. petersi and P. pustulosus* ("*P. pustulosus*" group) (Wassersug & Heyer, 1988; Spirandeli-Cruz,

TABLE 2. Summary of buccal floor variation for the species of *Physalaemus* in which buccopharyngeal morphology has been described. Abbreviations: PA, papillae; PU, pustulations.

"Species group"	Species	Lingual papillae				Prepocket papillae				Buccal floor arena
		1	2	3	0	1	1–2	2–3	6	
"*Physalaemus biligonigerus*" group	*P. biligonigerus* (Perotti & Céspedez, 1999)	X			X					8–10 PA, 30–35 PU
	P. santafecinus (Perotti & Céspedez, 1999)	X			X					
	P. fuscomaculatus (Nomura *et al.*, 2003)		X					X		8–12 PA, 30–40 PU
	P. nattereri (Spirandeli-Cruz, 1991)			X		X				>24 PA, several PU
"*P. pustulosus*" group	*P. petersi* (Wassersug & Heyer, 1988)	data unknown					X			data unknown
	P. pustulosus (Wassersug & Heyer, 1988)				X					12 PA, 20 PU
"*P. cuvieri*" group	*P. fernandezae*	X							X	6 PA,>60 PU

1991; Fabrezi & Vera, 1997; Perotti & Céspedez, 1999; Nomura *et al.*, 2003). No information on the buccopharyngeal morphology of species of the "*P. signiferus*" group is available. These features, in particular those from the buccal floor, are highly variable within some species groups (see Table 2). The buccal floor arena papillae and pustulations are putative characters for delimiting species groups within *Physalaemus*, but it would be interesting to know the range of variation for these characters.

On the other hand, other buccopharyngeal structures seem to be useful for the characterization of the "*P. biligonigerus*", "*P. cuvieri*" and "*P. pustulosus*" species groups: (1) species of the "*P. biligonigerus*" group possess four infralabial papillae; two semicircular arches of pustulations separated by a moderate notch and arranged in a V-shaped pattern on the prenarial arena; 6–8 conical papillae and 40–60 pustulations on the buccal roof arena; four postnarial papillae; a semicircular median ridge; and claw-shaped lateral ridges; (2) species of the "*P. pustulosus*" group are characterized by the possession of four infralabial papillae (shared with the "*P. biligonigerus*" group); four lingual papillae; two postnarial papillae; 12 conical papillae and 16–20 pustulations on the buccal roof arena; and short lateral ridges with rough and concave margins; (3) species of the "*P. cuvieri*" group differ from other species of the genus in having two infralabial papillae; three pustulations and two serrated papillae on the prenarial arena; three central and two lateral pustulations and two serrated papillae on the postnarial arena; four long and bifid papillae and up to 60 pustulations on the buccal roof arena.

The chondrocranium is known for *Physalaemus cuqui* ("*P. cuvieri*" group), *P. biligonigerus* ("*P. biligonigerus*" group), and *P. pustulosus* ("*P. pustulosus*" group) (Fabrezi & Vera, 1997; Larson & de Sá, 1998; Haas, 2003). Comparisons among these species and *P. fernandezae* allow a preliminary recognition of the following chondrocranial patterns within the genus: (1) the larval crista parotica is absent ("*P. cuvieri*" group), represented by a poorly developed anterior process ("*P. biligonigerus*" group), or possesses a well developed anterior process and a very reduced posterior process ("*P. pustulosus*" group); (2) closed commissura quadrato-orbitalis (*P. biligonigerus*, *P. cuqui* and *P. pustulosus*), or open (*P. fernandezae*); and (3) the processus anterolateralis hyalis of the ceratohyale may be reduced (*P. fernandezae*) or well developed (*P. cuqui*, *P. biligonigerus*). Larson & de Sá (1998) did not report this character for *Physalaemus pustulosus*.

The larval cranial musculature has been described for *Physalaemus cuqui* ("*P. cuvieri*" group), *P. biligonigerus* ("*P. biligonigerus*" group) and *P. pustulosus* ("*P. pustulosus*" group) (Starrett, 1968; Palavecino, 2000; Haas, 2003). The only difference among these species is that the muscle levator mandibulae lateralis is absent at advanced developmental stages in *P. fernandezae*, but it is present from stage 31 in the remaining species (Palavecino, 2000).

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REFERENCES

Alcalde, L. & Rosset, S. D. (2003). Descripción y comparación del condrocráneo en larvas de *Hyla raniceps* (Cope, 1862), *Scinax granulatus* (Peters, 1871) y *Scinax squalirostris* (A. Lutz, 1925) (Anura: Hylidae). *Cuadernos de Herpetología* **17**, 35–51.

Altig, R. & Johnston, G. F. (1989). Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. *Herpetological Monographs* **3**, 81–109.

Barrio, A. (1964). Relaciones morfológicas, eto-ecológicas y zoogeograficas entre *Physalaemus henselii* (Peters) y *P. fernandezae* (Muller) (Anura: Leptodactylidae). *Acta Zoologica Lilloana* **20**, 285–305.

Barrio, A. (1965). El género *Physalaemus* (Anura: Leptodactylidae) en Argentina. *Physis* **25**, 421–448.

Bokermann, W. C. A. (1962). Observaçoes biológicas sobre *Physalaemus cuvieri* Fitz., 1826 (Amphibia, Salientia). *Revista Brasileira de Biologia* **22**, 391–399.

Bokermann, W. C. A. (1963). Girinos de anfibios brasileiros 2 (Amphibia, Salientia). *Revista Brasileira de Biologia* **23**, 349–353.

Breder, C. M., Jr. (1946). Amphibians and reptiles of the Chucunaque drainage, Darien, Panamá, with notes on their life histories and habits. Bulletin of American *Museum of Natural History* **85**, 373–436.

Cannatella, D. C. & Duellman, W. E. (1984). Leptodactylid frogs of the *Physalaemus pustulosus* group. *Copeia* **4**, 902–921.

Caramaschi, U., Feio, R. N. & Guimarães-Neto, A. S. (2003). A new, brightly colored species of *Physalaemus* (Anura: Leptodactylidae) from Minas Gerais, Southeastern Brazil. *Herpetologica* **59**, 519–524.

Cardoso, A. J. & Haddad, C. F. B. (1985). Nova espécie de *Physalaemus* do grupo signiferus (Amphibia, Anura, Leptodactylidae). *Revista Brasileira de Biologia* **45**, 33–37.

Cei, J. M. (1980). Amphibians of Argentina. *Monitore Zoológico Italiano (Nova Serie) Monografia* **2**, 1–609.

Cruz, C. A. G. & Pimenta, B. V. S. (2004). New species of *Physalaemus* Fitzinger, 1826, from Southern Bahia, Brazil (Anura, Leptodactylidae). *Journal of Herpetology* **38**, 480–486.

de Beer, G. R. (1985). *The Development of the Vertebrate Skull.* Reprint, Chicago & London: University of Chicago Press.

D'Heursel, A. & de Sá, R. O. (1999). Comparing the tadpoles of *Hyla geographica* and *Hyla semilineata*. *Journal of Herpetology* **33**, 353–361.

Duellman, W. E. (1978). The biology of an equatorial herpetofauna in amazonian Ecuador. *University of Kansas, Museum of Natural History, Miscellaneous Publications* **65**, 1–352.

Duellman, W. E. (1997). Amphibians of La Escalera region, Southeastern Venezuela: taxonomy, ecology and biogeography. *Scientific Papers of the Natural History Museum, University of Kansas* **2**, 1–52.

Fabrezi, M. & Vera, R. (1997). Caracterización morfológica de larvas de anuros del noroeste argentino. *Cuadernos de Herpetología* **11**, 37–49.

Faivovich, J. (2002). A cladistic analysis of *Scinax* (Anura: Hylidae). *Cladistics* **18**, 367–393.

Fernandez, K. & Fernandez, M. (1921). Sobre la biología y reproducción de algunos batracios argentinos I. Cystignathidae. *Anales de la Sociedad Cientifica Argentina* **41**, 97–139.

Frost, D. R. (2004). *Amphibian Species of the World: An Online Reference. V2.21.* Electronic data base available at http://research.amnh.org/herpetology/amphibia/index.html.

Gallardo, J. M. (1963). Las especies bonaerenses de pequeño tamaño del género *Physalaemus* Fitzinger (Amphibia: Leptodactylidae). *Neotropica* **11**, 27–37.

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

Haas, A. (1995). Cranial features of dendrobatid larvae (Amphibia: Anura: Dendrobatidae). *Journal of Morphology* **224**, 241–264.

Haas, A. (2001). Mandibular arch musculature of anuran tadpoles, with comments on homologies of amphibian jaw muscles. *Journal of Morphology* **247**, 1–33.

Haas, A. (2003). Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics* **19**, 23–89.

Haas, A. & Richards, S. J. (1998). Correlations of cranial morphology, ecology, and evolution in Australian suctorial tadpoles of the genera *Litoria* and *Nyctimystes* (Amphibia: Anura: Hylidae: Pelodryadinae). *Journal of Morphology* **238**, 109–141.

Haddad, C. F. B. & Pombal JR., J. P. (1998). Redescription of *Physalaemus spiniger* (Anura: Leptodactylidae) and description of two new reproductive modes. *Journal of Herpetology* **32**, 557–565.

Haddad, C. F. B. & Sazima, I. (2004). A new species of *Physalaemus* (Amphibia: Leptodactylidae) from the Atlantic forest in Southeastern Brazil. *Zootaxa* **479**, 1–12.

Heyer, W. R., Rand, A. S., de Cruz, C. A. G., Peixoto, O. L. & Nelson, C. E. (1990). Frogs of Boracéia. *Arquivos de Zoologia, São Paulo* **31**, 231–410.

Johnston, G. F. & Altig, R. (1986). Identification characteristics of anuran tadpoles. *Herpetological Review* **17**, 36–37.

Kehr, A. I., Schaefer, E. F. & Dure, M. E. (2004). The tadpole of *Physalaemus albonotatus* (Anura: Leptodactylidae). *Journal of Herpetology* **38**, 145–148.

Langone, J. A. (1989). Descripción de la larva de *Physalaemus gracilis* (Boulenger, 1883) (Amphibia, Anura, Leptodactylidae). *Comunicaciones Zoológicas del Museo de Historia Natural de Montevideo* **12**, 1–11.

Langone, J. A. (1994). Ranas y sapos del Uruguay (reconocimiento y aspectos biológicos). *Museo Dalmaso A. Larrañaga, serie de divulgación* **5**, 1–118.

Larson, P. M. & de Sá, R. O. (1998). Chondrocranial morphology of *Leptodactylus* larvae (Leptodactylidae: Leptodactylinae): its utility in phylogenetic reconstruction. *Journal of Morphology* **238**, 287–305.

Lavilla, E. O. (1983). *Sistemática de Larvas de Telmatobiinae (Anura: Leptodactylidae)*. PhD thesis. Argentina: Facultad de Ciencias Naturales, Universidad Nacional de Tucumán.

Lobo, F. (1992). Descripción osteológica de *Physalaemus fernandezae* (Anura: Leptodactylidae) y comparación con otras especies del genero. *Acta Zoológica Lilloana* **XLII**, 51–57.

Lynch, J. D. (1970). Systematic status of the American leptodactylid frog genera *Engystomops*, *Eupemphix* and *Physalaemus*. *Copeia* **3**, 488–496.

Nascimento, L. B., Carvalho Jr., R. R., Wogel, H., Fernandes, D. S. & Feio, R. N. (2001). Reproducão e descripcão do girino de *Physalaemus rupestris* Caramaschi, Carcerelli and Feio, 1991 (Amphibia, Anura, Leptodactylidae). *Boletim do Museu Nacional (Nova Serie), Zoologia, Rio de Janeiro* **450**, 1–10.

Nomura, F., Rossa-Feres, D. & Mendonça do Prado, V. H. (2003). The tadpole of *Physalaemus fuscomaculatus* (Anura: Leptodactylidae), with a description of internal oral morphology. *Zootaxa* **370**, 1–8.

Palavecino, P. (2000). *Desarrollo de la Musculatura Mandibular e Hioidea en Leptodactylinae del Noroeste Argentino*. PhD thesis. Argentina: Facultad Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán.

Perotti, M. G. (1997). The tadpole of *Physalaemus cuqui* Lobo, 1993 (Amphibia, Anura, Leptodactylidae). *Amphibia–Reptilia* **18**, 427–432.

Perotti, M. G. & Céspedez, J. A. (1999). The tadpole of *Physalaemus santafecinus*, with comments on buccopharyngeal morphology. *Journal of Herpetology* **33**, 312–315.

Pimenta, B. V. S. & de Cruz., C. A. G. (2004). The tadpole and advertisement call of *Physalaemus aguirrei* Bokermann, 1966 (Amphibia, Anura, Leptodactylidae). *Amphibia–Reptilia* **25**, 197–204.

Pimenta, B. V. S., de Cruz, C. A. G. & Silvano, D. L. (2005). A new species of the genus *Physalaemus* Fitzinger, 1826 (Anura, Leptodactylidae) from the Atlantic rain forest of southern Bahia, Brazil. *Amphibia–Reptilia* **26**, 201–210.

Prigioni, C. M. & García, J. E. (2001). Descripción de la larva de *Physalaemus riograndensis* Milstead, 1960 (Anura: Leptodactylidae). *Acta Zoológica Platense* **1**, 1–5.

Ron, R. S., Cannatella, D. C. & Coloma, L. A. (2004). Two new species of *Physalaemus* (Anura: Leptodactylidae) from western Ecuador. *Herpetologica* **60**, 261–275.

Ron, R. S., Coloma, L. A & Cannatella, D. C. (2005). A new, cryptic species of *Physalaemus* (Anura: Leptodactylidae) from western Ecuador with comments on the call structure of the *P. pustulosus* species group. *Herpetologica* **61**, 178–198.

Rossa-Feres, D. C. & Jim, J. (1993). The tadpole of *Physalaemus centralis* (Anura: Leptodactylidae). *Copeia* **2**, 566–569.

Schlosser, G. & Roth, G. (1995). Distribution of cranial and rostral nerves in tadpoles of the frog *Discoglossus pictus* (Discoglossidae). *Journal of Morphology* **226**, 189–212.

Spirandeli-Cruz, E. F. (1991). *Estudo comparativo da Morfologia Oral Interna de Larvas de Anfibios Anuros que Occorem na Região de Botucatu, São Paulo (Amphibia: Anura)*. São Paulo: Instituto de Biosciências da Universidade de São Paulo.

Starrett, P. H. (1968). *The Phylogenetic Significance of the Jaw Musculature in Anuran Amphibians*. PhD. thesis. Ann Arbor, MI: University of Michigan.

Taylor, W. R. & Van Dyke, G. C. (1985). Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium* **9**, 107–119.

Van Dijk, D. E. (1966). Systematic and field keys to the families, genera and described species of Southern African Anuran tadpoles, with preliminary inclusion of certain undescribed and inadequately described tadpoles. *Annals of Natal Museum* **18**, 231–286.

Vizzoto, L. D. (1967). Desenvolvimiento de anuros da região norte occidental do estado de São Paulo. *Facultad de Filosofia, Ciencias e Letras. Departamento de Zoologia, (N° especial)*, 1–161.

Wassersug, R. J. (1980). Internal oral features of larvae from eight families: functional, systematic, evolutionary and ecological considerations. *Miscellaneous Publications, Museum of Natural History, University of Kansas* **68**, 1–146.

Wassersug, R. J. & Heyer, W. R. (1988). A survey of internal oral features of leptodactyloid larvae (Amphibia: Anura). *Smithsonian Contributions to Zoology* **457**, 1–99.

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GEOGRAPHIC VARIATION AND TAXONOMIC STATUS OF THE SOUTHERNMOST POPULATIONS OF *LIOPHIS MILIARIS* (LINNAEUS, 1758) (SERPENTES: COLUBRIDAE)

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We analyzed geographic variation in southern populations of *Liophis miliaris* and tested the hypothesis that *L. m. semiaureus* is a valid species. We examined 222 specimens from Argentina and Paraguay, including those from the areas of overlap of *L. m. semiaureus* and *L. m. orinus*, and compared these data with previous taxonomic revisions. We performed univariate statistical tests comparing *L. m. semiaureus* and *L. m. orinus*, and a discriminant function analysis using three morphological variables to compare four subpopulations, including two of *L. m. semiaureus* and two of *L. m. orinus*. We examined coloration in life in 152 specimens. These data and analyses support the hypothesis of *L. semiaureus* as a valid species: univariate analyses show significant differences in ventral and subcaudal numbers, and snout-vent length/tail length ratio between the two putative subspecies. *L. m. semiaureus* has significant more ventrals and subcaudals than *L. m. orinus*. Discriminant analysis separated two defined populations corresponding to *L. m. orinus* and *L. m. semiaureus*. Populations of *L. m. semiaureus* that are in contact with *L. m. orinus* populations show the highest ventral values of all of the *L. m. semiaureus* populations examined by us. We recorded differences in coloration among the juveniles of both subspecies, including specimens from neighbouring localities. The distributions are parapatric and have different ecological and historical settings. We discuss the validity of some diagnostic characters that have been used to distinguish *L. m. semiaureus*.

Key words: geographic variation, *Liophis miliaris semiaureus,* snake, taxonomy

INTRODUCTION

The colubrid snake *Liophis miliaris* (Linnaeus, 1758) has had a complex taxonomic history that has been summarized by Gans (1964) and Dixon (1983). Gans (1964) reviewed the morphological variation within the species, and although he discovered at least four "groupings", he refrained from naming subspecies, preferring to interpret the "groupings" as "samplings from the range of a single polymorphic species" (Gans 1964). However, because of the "peculiar interdigitation of ventral-count records along the northeastern edge of the high-count plateau" (i.e., southern Brazil, north-eastern Argentina, and southeastern Paraguay), Gans (1964) unsuccessfully attempted to find concordant characters that coincided with the high ventral counts of the southern population which might indicate that two replacing species were involved.

Dixon (1983) discussed the history of the holotype of *L. m. miliaris* and restricted it to Surinam, in northern South America, correcting Gans' (1964) assignation to Santos, São Paulo, Brazil. Additionally, Dixon (1983, 1989) included *L. amazonicus* (Dunn, 1922), *L. chrysostomus* (Cope, 1868) and *L. mossoroensis* Hoge & Lima-Verde 1972 as subspecies of *L. miliaris*. Furthermore, he proposed subspecies status for the four "groupings" discovered by Gans (1964), for a total of seven subspecies: *Liophis miliaris miliaris* Linnaeus, 1758, *L. miliaris chrysostomus* (Cope, 1868), *L. miliaris amazonicus* (Dunn, 1922), *L. miliaris mossoroensis* Hoge & Lima-Verde, 1972, *L. miliaris merremii* (Wied, 1821), *L. miliaris orinus* (Griffin, 1916), and *L. m. semiaureus* (Cope, 1862).

The subspecies of *Liophis miliaris* defined by Dixon (1983) have allopatric and parapatric distributions in distinct hydrological basins or subsectors in the greater South American basin (Dixon, 1983, 1989). In southern South America, *L. m. semiaureus* (type locality: "Paraguay", without more data, probably from the mouth of the Paraguay River according to Gans, 1964) shows remarkable morphological differences from the other forms, mainly in the number of ventrals and color pattern (Gans, 1964; Dixon, 1983). Dixon (1983) analyzed these differences, but supported the inclusion of these populations as subspecies of *L. miliaris*.

In this paper, we test the hypothesis that the populations of *L. m. semiaureus* (Cope, 1862) constitute a valid species separate from *L. miliaris*. We add new data, and discuss the diagnoses of Gans (1964) and Dixon (1983) for this taxon.

MATERIALS AND METHODS

We examined 222 specimens of *Liophis miliaris* from Argentina and Paraguay (see Appendix 1; Fig. 1). Standard methods for the study of snake taxonomy

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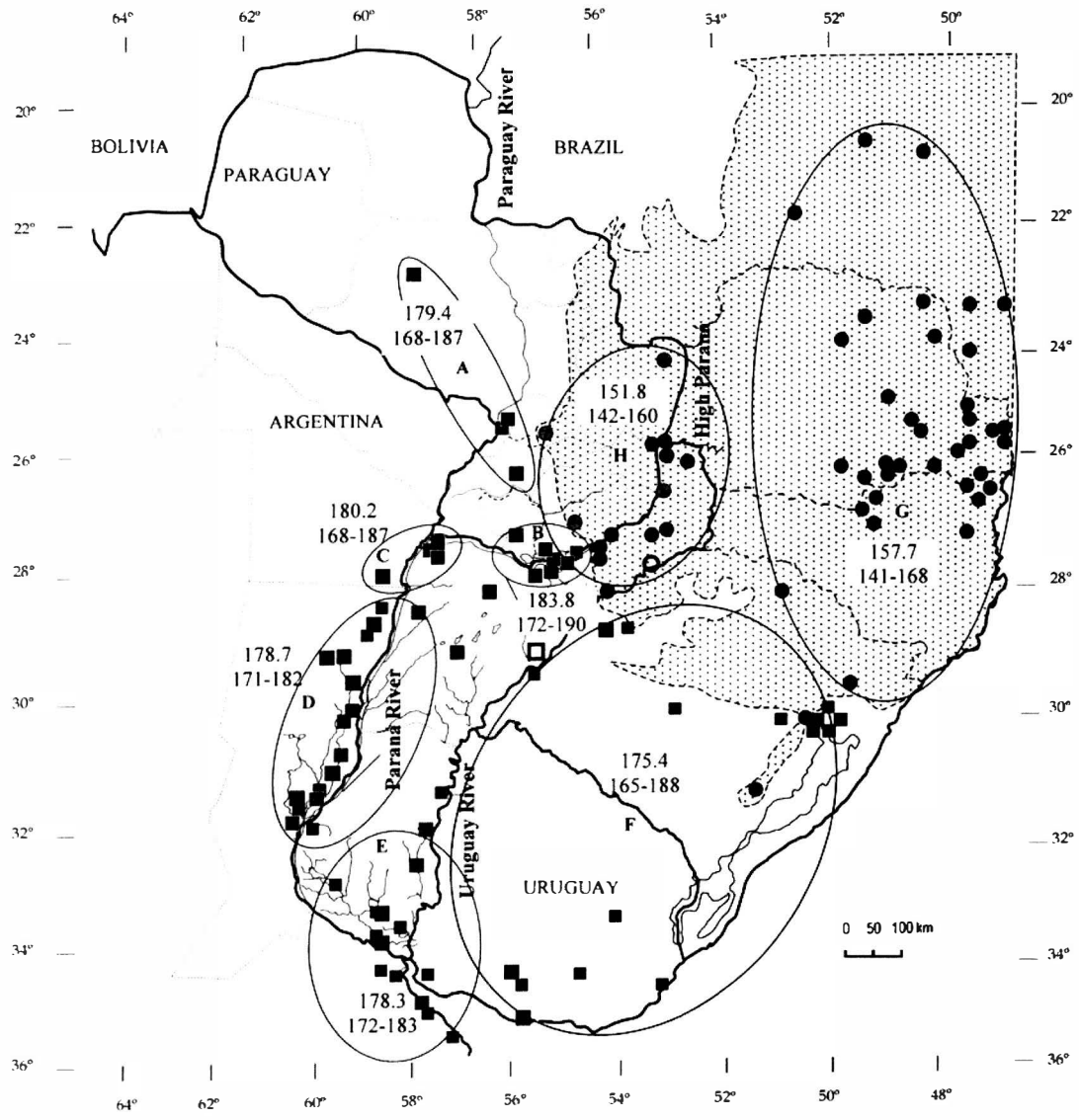

FIG. 1. Distribution of specimens of *Liophis m. semiaureus* (squares) and *L. m. orinus* (circles). Shaded area shows the Atlantic Forest Phytogeographic province (according to Galindo Leal & Camara 2003). The ventral number (mean above and range below) of *L. m. semiaureus* populations (A, B, C, D, E and F) and *L. m. orinus* populations (G and H) are indicated. The subpopulations of *L. m. semiaureus* from Argentina-Paraguay (A to E) and Uruguay-South of Brazil (F), and the subpopulations of *L. m. orinus* from Argentina-Paraguay (G) and Brazil (H) were used in discriminate function analysis (see methodology). The white circle and square show the localities of hatchlings specimens illustrated in Fig. 3.

were used. Ventral counts were made from the first scute wider than long, up to but not including the anal, following Gans (1964). Caudal counts were of pairs of segments, from the pair immediately anterior to the terminal spine, up to the last precloacal pair (Gans, 1964). Terminal spines were not included in subcaudal counts. Morphometric measurements were recorded using a ruler to the nearest millimetre for body and tail lengths. Snout-vent length (SVL) was measured from the tip of the snout to the posterior edge of the anal plate and tail length (TL) from the posterior edge of the anal plate to the tip of the tail. We used TL/SVL ratio given as percentage. We determined sex on specimens by making an incision posterior to the vent, and looking for with a dissecting scope the presence or absence of hemipenes. We examined living coloration in 152 adults and juveniles, and coloration in preservative in additional 70 specimens.

The univariate normality assumptions of numerical characters were verified using Shapiro-Wilks test, while homogeneity of variance was verified with the *F*-test. We performed parametric (Student *t*) and non-parametric (Mann-Whitney) univariate statistical tests (depending on the normality and homogeneity of the variables) to compare sexual dimorphism and population differences among *Liophis miliaris* examined by us.

We performed a simple linear correlation on ventral number against latitude for all *Liophis miliaris* examined by us and for each subspecies to determine whether clinal variation exists. The expected cline of ventral variation is that the northern populations have the low-

est ventral numbers (*L. m. orinus*) and the southernmost populations have the highest ventral numbers (*L. m. semiaureus*).

Additionally, we performed a discriminant function analysis comparing four subpopulations, including two of *L. m. semiaureus* (Paraguay-Argentina and Uruguay-South Rio Grande do Sul state, Brazil) and two of *L. m. orinus* (Paraguay-Argentina and north Rio Grande do Sul to Minas Gerais, Brazil; Fig. 1), using ventral and subcaudal numbers, and TL/SVL ratio. Other measurements were excluded to avoid covariant characters. We used the Gans'(1964, Table 1) data for subpopulations of *L. m. semiaureus* from Uruguay and south of Rio Grande do Sul state in Brazil (n=62) and *L. m. orinus* from north of Rio Grande do Sul, Santa Catarina, Paraná, São Paulo and Minas Gerais states in Brazil (n=291), following Dixon's (1983) subspecific assignation and distribution.

All statistical analyses were performed using Infostat (2002) version 1.6 with significance level of 0.05.

RESULTS

SCALATION AND MEASUREMENT CHARACTERS

Although all comparisons yielded the normal colubrid pattern wherein males average fewer ventrals

TABLE 1. Variation and sexual dimorphism of some characters in *Liophis miliaris* southern populations examined by us. Abbreviations: F, female; M, male; n, sample size; SD, standard deviation; range: minimum and maximum values; U, Mann-Whitney statistic value.

Characters	Subspecies	Sex	n	Mean	SD	Range		Statistical comparison
Ventrals	*orinus*	F	16	152.2	4.89	143	160	U=213
		M	14	151.3	4.91	142	158	P=0.4401
	semiaureus	F	118	182.2	4.25	171	190	U=6886
		M	74	181.4	4.62	168	190	P=0.32
Subcaudals	*orinus*	F	15	52.9	3.26	47	61	U=244
		M	14	55.2	4.58	50	67	P=0.1348
	semiaureus	F	103	57.8	3.71	43	65	U=5856.5
		M	66	58.5	3.19	50	68	P=0.4244
Total length (TOL)	*orinus*	F	15	514.9	284.41	175	906	U=204.00
		M	14	432.5	215.69	208	798	P=0.3708
	semiaureus	F	101	764.8	266.57	234	1457	U=4379.50
		M	66	619.5	151.52	199	967	P<0.0001
Tail length (TL)	*orinus*	F	15	94.4	48.65	33	164	U=204.50
		M	14	85.4	41.89	38.7	154	P=0.3824
	semiaureus	F	101	127.72	41.6	35	220	U=4374.5
		M	68	107.8	27.33	35	164	P<0.0003
Snout-vent length (SVL)	*orinus*	F	17	445.9	242.23	142	804	U=206.5
		M	14	347.1	175.24	168	644	P=0.1647
	semiaureus	F	117	660.9	232.02	198	1259	U=5305.5
		M	73	517.8	125.96	164	803	P<0.0001
TL/SVL	*orinus*	F	15	22.7	1.83	20	26	U=258.5
		M	14	24.8	3.40	20	35	P=0.0314
	semiaureus	F	100	20.3	1.86	16	25	U=6530
		M	65	21.4	1.61	17	26	P=0.0001

TABLE 2. Univariate analysis comparing *L. m. orinus* and *L. m. semiaureus* subspecies. Abbreviations: n, sample size; SD, standard deviation; U, Mann-Whitney statistic.

	L. m. orinus			*L. m. semiaureus*			Statistical comparison	
Characters	n	Mean	SD	n	Mean	SD	U value	P value
Ventrals	31	151.81	4.76	193	181.89	4.41	496	<0.0001
Subcaudals	30	54.03	3.98	170	58.05	3.52	1379.5	<0.0001
Total length	30	486.5	255.92	170	702.81	240.31	1885.5	0.0001
Tail length	30	91.89	45.27	169	119.08	37.93	2148.5	0.0043
Snout-vent length	32	409.76	218.8	193	604.28	213.09	2217.5	0.0001
TL/SVL	30	23.67	2.82	168	20.93	5.09	4627.5	<0.0001

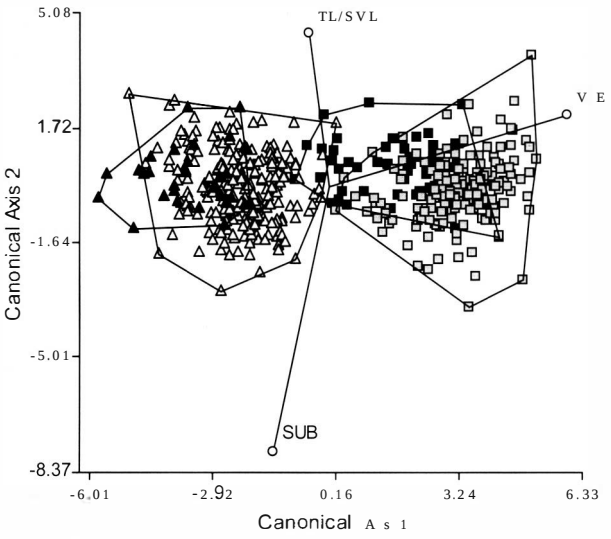

FIG. 2. Biplot of discriminant analysis among morphological variables (VE: Ventrals, SUB: Subcaudals, TL/SVL: Tail length/Snout-vent length ratio) of *L. m. orinus* (Argentina-Paraguayan populations black triangles and Brazilian populations shaded triangles) and *L. m. semiaureus* (Argentina-Paraguayan populations shaded squares and Uruguayan-Brazilian populations black squares (see Fig. 1).

and more subcaudals than females, none of the differences were significant. All measurements showed significant sexual dimorphism in *L. m. semiaureus* but not in *L. m. orinus*, with exception of TL/SVL, in which males had relatively longer tails in both taxa (Table 1). Females of *L. m. semiaureus* averaged significantly longer than males in all measurements (Table 1).

The univariate analyses showed significant differences in all characters examined between the two subspecies (Table 2). Ventral numbers did not overlap between the subspecies in specimens examined by us, with a very significantly higher number of ventrals in *L. m. semiaureus*. The ventrals (U=114682.5, P<0.0001), subcaudals (U=64287, P<0.0001) and TL/SVL ratio (U=32599.5, P<0.0001) showed significant differences between the two subspecies when combining our data with Gans' data. The numbers of ventral for all these sample is 141 to 168 (mean 157.1) for *L. m. orinus* (n= 322), and 165 to 190 (mean 180.3), for *L. m. semiaureus* (n= 255).

The discriminant analyses showed that *L. m. orinus* subpopulations are separated from the *L. m. semiaureus* subpopulations with a small degree of overlap (Fig. 2). The subpopulations of *L. m. orinus* from Paraguay-Argentina (our data) and Brazil (Gans' data) were grouped among them. *L. m. semiaureus* subpopulations from Paraguay-Argentina (our data) and Uruguay-South Brazil (Gans' data) were also grouped (Fig. 2). Most of the variation of canonical discriminant axis 1 is explained by number of ventral scales while most of the variation of axis 2 is explained by number of subcaudal scales (Table 3).

TABLE 3. Results of discrimant analysis of three morphological variables and four subpopulations two of *L. m. orinus* and two *L. m. semiaureus* (see Fig. 1).

	Canonical axis 1	Canonical axis 2
Ventrals	1.05	0.37
Subcaudals	-0.25	-1.36
TL/SVL	-0.09	0.79

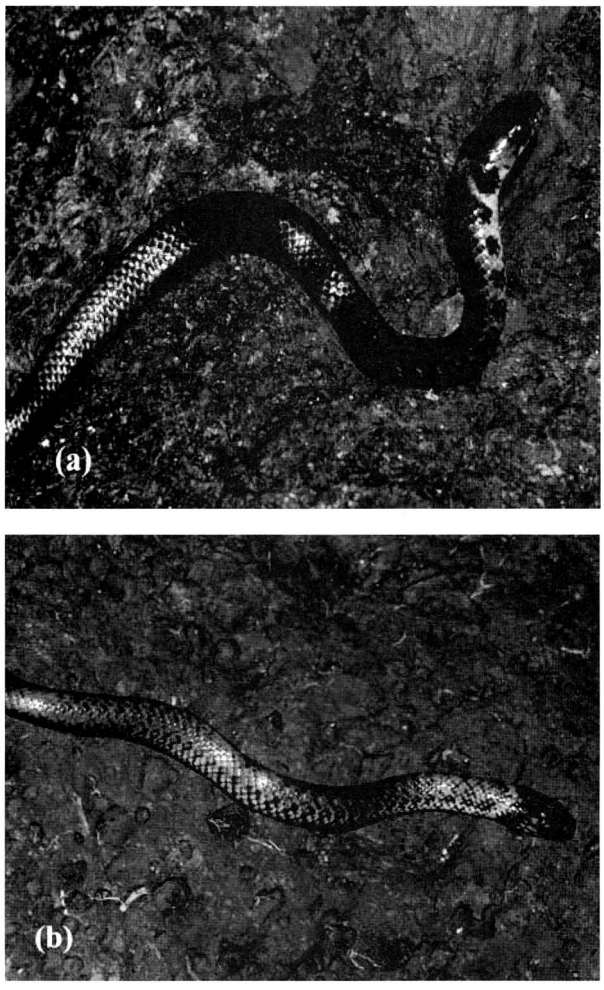

FIG. 3. Hatchlings specimens of *L. miliaris orinus* (206 mm TOL) (a) and *L. miliaris semiaureus* (244 mm TOL) (b), both with umbilical scars, caught between 30 and 28 January of 2000, respectively, from two localities separated by 170 km airline (see Fig. 1). Photos by A. Giraudo.

The correlation between ventral numbers and latitude for *Liophis miliaris* examined by us was not significant (Spearman correlation, r=-0.05591, n=219, P=0.4104), and *L. m. semiaureus* populations showed a lower negative significant correlation between ventral numbers and latitude (Spearman correlation, r_s=-0.45, n= 194, P<0.0001). Populations of *L. m. semiaureus* that are in contact with *L. m. orinus* populations show the highest ventral values of all of the *L. m. semiaureus* populations examined by us (Fig. 1).

COLORATION

The two subspecies show differences in coloration and pattern. Both have a pattern called "salt and pepper" by Peters & Orejas Miranda (1970), with dorsal scales having light bases and dark free margins, giving

TABLE 4. Summary of characters of *Liophis miliaris* subspecies. Modified from Dixon (1983), with addition of our data marked in bold.

Subspecies	Ventrals			Subcaudals			TL/TOL		
	N	Mean	Range	*N*	Mean	Range	*N*	Mean	Range
L. miliaris amazonicus	6	155.2	148-159	2	75.5	75-76	No data		
L. miliaris chrysostomus	63	159.4	153-172	49	59.5	53-64	31	19.35	16.8-21
L. miliaris merremii	126	146.5	135-156	112	49.5	40-56	65	18.5	15-22.2
L. miliaris miliaris	19	156	142-163	15	57	51-64	10	18.9	17.6-20
L. miliaris mossoroensis	23	158.7	148-166	23	50.8	45-56	23	17	15.3-18.5
L. miliaris orinus	302	158.4	147-169	246	56.2	47-69	203	19.06	15-22.4
L. miliaris orinus	**31**	**151.81**	**142-160**	**30**	**40.3**	**47-67**	**30**	**19.17**	**17-26**
L. miliaris semiaureus	100	176.2	163-190	84	56.7	50-68	79	17.91	5.7-20.5
L. miliaris semiaureus	**193**	**181.89**	**168-190**	**170**	**58.05**	**43-68**	**168**	**17.24**	**13-21**

the overall appearance of an olive-green snake. In *L. m. semiaureus* the general coloration is lighter because the dark line that borders the distal scale edge is narrower than in *L. m. orinus*. In *L. m. orinus* the dark colour extend to the center scale (Giraudo, 2001: Plate 14). In *L. m. semiaureus*, the midbody and posterior ventral plates have a continuous dark line along the entire distal scale border. In *L. m. orinus*, this dark line is narrower and generally discontinuous or very faded in the central and posterior portion of the belly. The general adult colour pattern is more regular and has less variation in *L. m. semiaureus* than in *L. m. orinus*.

The coloration of the hatchlings of *L. m. orinus* coincides with the description and figures shown by Gans (1964), and is characterized by a thin light nuchal V-shaped collar (white in fixed and yellow in live specimens) that is one or two dorsal scales wide, bordered by a parallel anterior V-shaped dark parietal-occipital band and by a parallel posterior wider V-shaped dark band (black in fixed and live specimens) occupying three to four dorsal scales. This black-light-black nuchal collar is followed by a well-defined series of dark dorsal and lateral blotches, more developed in the anterior part of the body. These have a tendency to coalescence towards the tail (Fig. 3a), forming a lateral black caudal stripe. In contrast, in hatchlings of *L. m. semiaureus*, the black-light-black nuchal collars are very narrow and poorly defined, and the dark lateral and dorsal blotches are poorly developed, consisting of a series of small spots that are very reduced posteriorly (Fig. 3).

DISCUSSION AND CONCLUSIONS

Gans (1964) described the body coloration of juveniles from the southern, high-ventral count population as being relatively uniform, not showing the alternating dark and light zones found in more northern populations. Additionally, these juveniles had smaller and more subdued dark markings. However, later in the same paper, Gans (1964) also described and illustrated the striking retention of juvenile blotches in several adult specimens from near Montevideo and Buenos Aires.

We cannot corroborate Gans' (1964) statement (followed by Dixon 1983) that *L. m. semiaureus* retains a juvenile blotched pattern in the populations of Argentina and Paraguay. Several specimens of *L. m. semiaureus* between 257 and 449 mm of total length (TOL) and all larger specimens have the adult coloration pattern. On the contrary, the *L. m. orinus* examined retained the juvenile colour pattern in older specimens including several adults, such as FML 975 (647 mm TOL), which retained vestiges of a light nuchal collar and dark lateral blotches on the anterior body.

Dixon (1983), probably following Gans (1964), indicated in the diagnosis of *L. m. semiaureus* "pronounced retention of dorsal blotch patterns in adults". We have not found adults of *L. m. semiaureus* with retained juvenile blotch patterns in a large sample from Argentina and Paraguay (n=193), including several specimens collected in the surroundings of Buenos Aires (in the MACN and MLP collections). We consider that the retention of juvenile is atypical within *L. m. semiaureus* in the populations of Argentina and Paraguay (the type locality being in the latter country), and therefore this coloration is not a diagnostic character for the taxon. However, according to Gans (1964) and Achaval and Olmos (2003), we observed the retention of juvenile patterns in 10 of 21 specimens of *L. m. semiaureus* (48%) from Uruguay populations.

Dixon (1983) indicated in the diagnosis of *L. m. semiaureus* a range of 163 to 190 ventrals with a mean of 176.2 (n=100). We found a similar range (168 to190) but our mean (181.9, n=193) and the minimum value are slightly higher than Dixon's. We consider that these minimal differences probably are due to that Dixon (1983) included in his material of *L. m. semiaureus* two specimens from Montecarlo, near Iguazú Falls (province of Misiones), which were not examined by us but we suspect to be *L. m. orinus* as all material examined by us from the area of Montecarlo corresponds to *orinus*, and this locality is in the Interior Atlantic Forest of Argentina (Giraudo 2001), well off the range of *L. m. semiaureus*.

In the total sample of 255 specimens of *L. m. semiaureus* (range 165 to 190, mean of 180.3 including

our data and Gans' data) only 14 overlap slightly with the maximum values of ventrals of *L. m. orinus* (one from Argentina, one from Paraguay, six from Uruguay and six from Rio Grande do Sul Brazil), with values between 165 to 168 ventrals. These specimens could be considered intermediates between the two subspecies, although another possible explanation lies in the large number of snakes that are rafted down the Paraguay-Paraná and the Uruguay river systems in floating mats of water hyacinths (Achaval *et al.* 1979). For this reason, these snakes could be also considered *L. m. orinus* that have come down from the upper parts of the river system. On the other hand, in the total sample of 322 specimens of *L. m. orinus* (141 to 168 ventrals, mean of 157.1) only 19 overlap slightly with the minimum values of ventrals of *L. m. semiaureus* (165 to 168), but notably all they are of areas relatively far from the subspecies contact zone (14 of São Paulo state, two of Santa Catarina state and two of Paraná state in Brazil). We considered that probably these are extreme values of ventrals included in the variation of the *L. m. orinus* subspecies.

The following data and analyses support that *Liophis. m. semiaureus* is a valid species:

(1) Specimens of *L. m. semiaureus* have higher number of ventrals than any *Liophis miliaris* (Table 4), without significant overlap, and this difference is very significant with respect to the contiguous populations of *L. m. orinus*. Other characters show the same pattern, according to both univariate and multivariate analyses (see results and Table 1).

(2) There is no clinal variation in the number of ventrals, supported by an insignificant correlation between ventrals and latitude in all examined specimens, and by a significant negative correlation in *L. m. semiaureus.*

(3) The closest population of *L. m. semiaureus* to *L. m. orinus* show the highest ventral scale counts of all the *L. m. semiaureus* populations examined by us (see population B in Fig. 1). These populations of both subspecies are separated by only 40 km along the same river and they are connected by the Paraná River. A large number of snakes are swept down in seasonal floodings of this river in floating mats of water hyacinths called "camalotales" (e.g. Achaval *et al.* 1979). In spite of this, we did not record any intermediate specimens in this area. This is indirect evidence of null or low genetic exchange between these populations. Gans (1964) observed the same pattern as he included a line in the map of his Figs. 8 and 10 representing the region where an abrupt jump takes place in the number of ventrals of *Liophis miliaris*. Gans (1964) stated that "It is interesting to note that the low-count plateau seems to send a finger south into Rio Grande do Sul, as the single specimen from Cerrito a low count in contrast to the high count of specimens in the adjacent localities in Pelotas and Rio Grande do Sul". Cerrito is located in southeastern hills of Rio Grande do Sul, Brazil, considered one of the most southern areas of Atlantic biogeographical influence (Galindo & Câmara, 2003; Lema, 1994), while Pelotas is in adjacent lowlands that have a Pampean biogeographical influence (Lema, 1994).

(4) We recorded differences in coloration among the juvenile of both subspecies, including specimens of nearby localities (170 airline-km) (see results and Fig. 3).

(5) The subspecies *L. m. orinus* inhabit the Atlantic biogeographical province in hills, plateaus and mountains of eastern Paraguay, northeastern Argentina and southeastern Brazil (Fig. 1). This area is dominated by Atlantic rainforest, and the main aquatic habitats are streams and rivers characterized by basaltic channel with rapid stream and a narrow flood valley. The subspecies *L. m. semiaureus* inhabit lowlands of Chaco-Pampean plain in great floodplain rivers with extensive alluvial valleys forming several lentic aquatic habitats such as marshes and lagoons. Their distributions are parapatric in areas with different ecological and historical settings.

We conclude that *L. semiaureus* is a well defined taxon. The possible existence of a few intermediates specimens in ventral counts did not invalidate our conclusions. We follow the evolutionary species concept postulated by Wiley (1978) and discussed by Frost & Hillis (1990), Frost *et al.* (1992) and Giraudo (1997), frequently used in herpetology (e.g., Collins, 1991; Reichling, 1995). In light of this concept *Liophis semiaureus* is undoubtedly a valid species.

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REFERENCES

Achaval, F. & Olmos, A. (2003). *Anfibios y reptiles del Uruguay*. Montevideo: Graphis Impresora.

Achaval, F., Gonzalez, J. G., Meneghel, D. M. & Melgarejo, A. R. (1979). Lista comentada del material recogido en costas uruguayas, transportado por camalotes desde el Río Paraná. *Acta Zoologica Lilloana* **35,** 195–200.

Collins, J. T. (1991). Viewpoint: a new taxonomic arrangement for some North American amphibians and reptiles. *Herpetological Review* **22**, 42–42.

Dixon, J. R. (1983). Taxonomic status of the South American snakes *Liophis miliaris*, *L. amazonicus, L. chrysostomus, L. mossoroensis* and *L. purpurans*. *Copeia* **1983**, 791–802.

Dixon, J. R. (1989). A key and checklist to the neotropical snake genus *Liophis*, with country list and maps. *Smithsonian Herpetoogical Information Service* **79**, 1–40.

Frost, D. R. & Hillis, D. M. (1990). Species in concept and practice: herpetological applications. *Herpetologica* **46**, 87-104.

Frost, D. R., Kluge, A. G. & Hillis, D. M. (1992). Species in contemporary herpetology: comments on phylogenetic inference and taxonomy. *Herpetological Review* **23**, 46–54.

Galindo Leal, C. &. Câmara, I. G. (2003). *Atlantic Forest Hotspots Status: An Overview*. In *Atlantic Forest of South America. Biodiversity status, threats, and outlook*, 3-11. Galindo-Leal, C. and Câmara, I.G. (Eds.). Washington: Island Press.

Gans, C. (1964). A redescription of, and geographic variation in, *Liophis miliaris* Linné, the common water snake of southeastern South America. *American Museum Novitates* **2178**, 1–58.

Giraudo, A. R. (1997). El concepto de especie. Parte I. *Natura Neotropicalis* **28,** 35–44.

Giraudo, A. R. (2001). *Diversidad de serpientes de la Selva Paranaense y del Chaco Húmedo. Taxonomia, biogeografía y conservación*. Buenos Aires: Literature of Latin America.

Lema, T. (1994). Lista comentada dos répteis ocorrentes no Rio Grande do Sul, Brasil. *Comunicações do Museu de Ciencias e Tecnologia da PUCRS, Série Zoologia* **7**, 41–150.

Peters, J. R. &. Orejas Miranda, B. (1970). Catalogue of the neotropical squamata: Part I. Snakes. *Bulletin of the United States National Museum* **297**, 1–347.

Reichling, S. V. (1995). The taxonomic status of the Louisiana Pine Snake (*Pituophis melanoleucus ruthveni*) and its relevance to the evolutionary species concept. *Journal of Herpetology* **29**, 186-198.

Wiley, E. O. (1978). The evolutionary species concept reconsidered. *Systematic Zoology* **27**, 17–26.

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APPENDIX I

SPECIMENS EXAMINED

Museum abbreviations are: CENAI: Centro nacional de Investigaciones Iológicas collection, actually deposited in MACN, Buenos Aires; CCP: Colección privada Caa Porá, Iguazú, Misiones; CIES: Centro de Investigaciones Ecológicas Subtropicales, Parque Nacional Iguazú, Misiones; CUNAM: Universidad Nacional de Misiones, Posadas, Misiones; FML: Fundación Miguel Lillo, Tucumán; MACN: Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires; MLP: Museo de La Plata, La Plata, Buenos Aires; MNHNP: Museo Nacional de Historia Natural del Paraguay, San Lorenzo; UNNEC: Universidad Nacional del Nordeste, Corrientes.

Liophis miliaris orinus. ARGENTINA: Misiones Province: Cainguás Department: 2 de Mayo CENAI 314, 3319, Balneario Arroyo Cuña Piru CFA 801; Candelaria Department: Santa Ana CENAI 3349; Gral. M. Belgrano Department: Deseado, Arroyo Bruaca CCP 30- 31; Arroyo San Jorge MACN 34627; Eldorado Department: Eldorado MACN 28008-28009; Iguazú Department: Parque Nacional Iguazú, Barrio de Guardaparques CIES 10, 54; Parque Nacional Iguazú CIES 21, 25, 68, 206, 222; Parque Nacional Iguazú, acceso a Puerto Canoas CIES 43; Puerto Iguazú INALI 1203- FML 205; 25 de Mayo Department: 25 de Mayo INALI 1053; El Bonito FML 676-1- 676-2; San Ignacio Department: Gobernador Roca CFA 58; Arroyo El Rosario, 25 Km de San Pedro FML 975; PARAGUAY: Cordillera Department: Itacurubí de la Cordillera MNHNP 2607; Canindeyu Department: 11 Km S de Katuete MNHNP 3777-3835; Itapúa Department: Itapúa, Arroyo Tingazú, 52 km NW of Pirapó MNHNP 8863; Paraguay without precise localities MNHNP 8787 MNHNP 9497

Liophis miliaris semiaureus. ARGENTINA: Buenos Aires Province: Capital Federal: Reserva Costanera Sur MACN 34559; Ensenada Department: Punta Lara MACN 31979, 31980 , 31981, 31982; Punta Lara CENAI 2945; Magdalena Department: Magdalena CENAI 3199, 3200; Luján Department: Dique Luján MACN 34644; San Pedro Department: Vuelta de Obligado MACN 36080; Isla Giovannini, Río Barca Grande, Delta bonaerense MACN 29956; Delta Bonaerense MACN 25071; Quilmes Department: Quilmes MACN 864; Corrientes Province: Capital Department: Laguna Pampín, Barrio Las Lomas CFA 111, 195, 214, 222, 243, 247, 257, 292, 567, 756, 788, 789; Laguna Soto CFA 30; Corrientes, Barrio Santa Rita CFA 146; Granja Yatay CFA 741, 742; CAPRIM, San Cayetano CFA 790; 10 km S of Loreto, nacional route 12 CFA 326; Mercedes Department: Mercedes CENAI 3443; Ituzaingó Department: Isla Oculto UNNEC 470; 23 km S of Santo Tomé, nacional route 14 INALI 1047; national route 12 INALI 44; Entre Ríos Province: Concordia Department: Concordia, 12 km W of Colonia Roca INALI 1104; Colón Department: Arroyo Barú MACN 30694; Victoria Department: 1 km S of Rincón de Nogoya, provincial route 11 INALI 1517; Islas del Ibicuy

Department: 2 km N of Médanos, provincial route 12 INALI 1489; 11 km NW of Médanos, provincial route 12 INALI 1512; Villa Paranacito INALI 1550; 37 km E of Puerto Ibicuy INALI 1559,1560; 40 km E of Puerto Ibicuy INALI 1562; 3 km al NE of Villa Paranacito INALI 1551. Misiones Province: Capital Department: Posadas, Puente internacional CUNAM 179; Nemesio Parma CUNAM 309; Posadas, Arroyo El Zaimán CUNAM 316; Posadas CUNAM 340, 341; Santa Fe Province: Garay Department: 12 km N of Santa Rosa, provincial route 1 (31° 21´S - 60° 15´O) INALI 187; 11 km N of Santa Rosa, provincial route 1 (31° 22´S - 60° 17´O) INALI 193; 4 km N of Colonia San Joaquín, provincial route 1 INALI 504; 4 km N of Helvecia, provincial route 1 INALI 610; Saladero Cabal, provincial route 1 INALI 614; 13 km S of Cayastá, provincial route 1 INALI 738; Vuelta del Dorado, Cayastá INALI 835; 8 km S of Santa Rosa de Calchines, provincial route 1 INALI 1293; 5 km S of Cayastá, provincial route 1 INALI 1383; 8 km S of Cayastá, provincial route 1 INALI 1627; 8 km S of Santa Rosa, provincial route 1 INALI 1630; 2 km S of Santa Rosa, provincial route 1 INALI 999; General Obligado Department: Arroyo Ceibal, nacional route 11 INALI 217, 223; El Rabón INALI 688; La Capital Department: 8 km S of Laguna Paiva, provincial route 2 INALI 989; Santo Tomé INALI 1079; Arroyo Potrero INALI 1292; 3 km N Ascochinga, provincial route 2 INALI 1597; 5 km N of Rincón INALI 1611; 4 km N of Arroyo Leyes INALI 1612; Santa Fe INALI 1632; San Javier Department: 16 km N of San Javier, provincial route 1 INALI 966; 40 km N of Alejandra, provincial route 1 INALI 978; 36 km N of San Javier, provincial route 1 INALI 1275; 19 km N of Alejandra provincial route 1 INALI 1525; Vera Department: 4 km E of Vera, provincial route 98 INALI 639; 14 km E of Vera, provincial route 98 INALI 645; BRAZIL: Rio Grande do Sul state: Estación Ecológica TAIM, Brasil, Nelson Perez CFA 434; PARAGUAY: Central Department: Areguá MNHNP 6573, 6574, San Lorenzo MNHNP 2527; Itapúa Department: Isla Cururú MNHNP 4605, 4606, 4607, 4608, 4613, 4614, 4615, 4616, 4617, 4618, 4619, 4620, 4666, 4667, 4668, 4669, 4670, 4671, 4672, 4673, 4674, 4675, 4676, 4677, 4678, 4680, 4681, 4683, 4684, 4685, 4686, 4687, 4688, 4689, 4690, 4691, 4692, 4693, 4695, 4696, 4697, 4698, 4699, 4700, 4701, 4702, 4703, 4704, 4705, 4706, 4707, 4708, 4709, 4710, 4711, 4712, 4713, 4714, 4715, 4716, 4717, 4719, 4720, 4751, 4752, 4753, 4754; Isla Guasú'i MNHNP 4811,4812, 4837, 8350; Isla Modesto MNHNP 4750, 4939; Isla Paloma MNHNP 6591, 8338, 8365 Isla Talavera MNHNP 4609, 4610, 4611, 4612, 4744, 4745, 4746, 4747, 4748, 4749, Isla Yacyreta MNHNP 4810, 4839, 4861, 4862, 4863, 4864, 4865, 4961, 6705, 6710, 6712, 6713, 7947, 8356, 8357, 8360, 8364, 9164; Isla Yacyreta, José Cué MNHNP 4838; Misiones Department: Ruta San Ignacio-Yabebyry Km 70 MNHNP 9145; Paraguari Department: 26°06'27"S; 56°56'38"O MNHNP 6749; Presidente Hayes Department: Estancia Salazar, km 340 route "Trans Chaco" MNHNP 6638.

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GENETIC EVIDENCE FOR TWO DISTINCT SPECIES WITHIN THE ITALIAN ENDEMIC *SALAMANDRINA TERDIGITATA* (BONNATERRE, 1789) (AMPHIBIA: URODELA: SALAMANDRIDAE)

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Genetic variation in 12 populations of the Italian endemic spectacled salamander *Salamandrina terdigitata* was investigated through the analysis of 29 allozyme loci. Two well-differentiated population groups were identified, one ranging from the Tusco-Emilian Apennine to southern Latium, the other comprising populations from central Campania to Calabria. Nine diagnostic and four highly differentiated loci led to an average genetic distance of D_{Nei}=0.47 between the two groups, while within them D_{Nei} ranged from 0.00 to 0.05. The observed genetic structure strongly suggests that two distinct species have so far been included within *Salamandrina terdigitata*. The names *Salamandrina perspicillata* (Savi, 1821) and *S. terdigitata* (Bonnaterre, 1789) are here proposed for the species from central and southern Italy respectively.

Key words: Caudata, cryptic species, Italy, molecular taxonomy

INTRODUCTION

Since their emergence, biochemical and molecular techniques have allowed the study of the genetic structure of populations, providing evidence for the existence of cryptic biodiversity that was previously unsuspected. For amphibians, which are generally conservative in their morphological evolution (Cherty *et al.*, 1978; Hass *et al.*, 1995; Richards & Moore, 1996), the routine use of these tools has led to the identification of an astonishing number of morphologically "cryptic" species (e.g. Duellman, 1993; Nascetti *et al.*, 1996; Hanken, 1999; Frost, 2002), even in the well-studied European batrachofauna, as recently reviewed by Veith (1996) and Borkin (1999). In fact, the number of amphibian species recognized for the European area has almost doubled during the last four decades (Mertens & Wermuth, 1960; Frost, 2002), but it should be borne in mind that several species have not yet been investigated. Among these, *Salamandrina terdigitata* constitutes an interesting case study. It is a stream-breeding species endemic to peninsular Italy, mainly distributed on the western side of the Apennine chain from 200 m to 900 m a.s.l. (Lanza, 1983; Mazzotti *et al.*, 1999; Corsetti & Angelini, 2000; see Fig. 1). It is the only known representative of a genus which, according to Titus & Larson (1995), is an ancient lineage that separated from other newt lineages very shortly after the split between newts and true salamanders. The species is protected by international and regional laws (it is listed in Annexes II and IV of the EU Council Directive for the Conservation of Natural Habitats and of Wild Fauna and Flora), and it is of particular concern to Italian zoologists because it is the only Italian endemic terrestrial vertebrate genus (Lanza, 1988). In spite of this, its geographic variation and genetic population structure have been investigated only recently (Nascetti *et al.*, 2005). According mainly to mitochondrial DNA sequence data, that study suggested the existence of two distinct species within the spectacled salamander. However, the use of cytoplasmic markers (i.e. mitochondrial or chloroplast DNA) alone to recognize species has been criticized, mainly because in diverging populations they become reciprocally monophyletic much faster than even a single nuclear locus, and very much faster than a set of nuclear loci (e.g. Hudson & Coyne, 2002). Therefore, in this paper we provide further data on the genetic population structure of the spectacled salamander, as assessed by means of 29 nuclear (allozyme) loci.

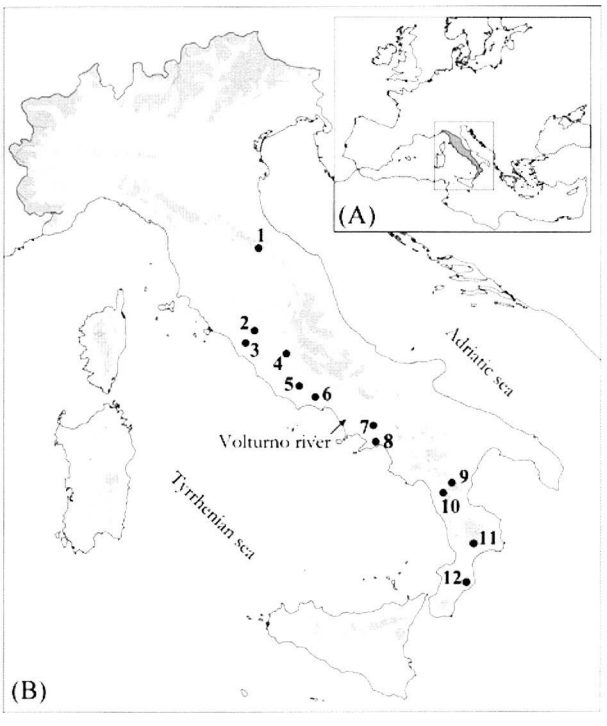

FIG. 1. Species range (A) and geographic location of the 12 populations of *S. terdigitata* sampled (B). Localities are numbered as in Table 1.

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TABLE 1. Geographic origin and sample size (*n*) of the 12 populations of *S. terdigitata* studied.

Sample code	Locality	Altitude (m a.s.l.)	Region	*n*
1	Bagno di Romagna	460	Emilia-Romagna	20
2	Barbarano Romano	340	Latium	9
3	Tolfa	480	Latium	7
4	Percile	575	Latium	11
5	Bassiano	560	Latium	12
6	M.te San Biagio	140	Latium	10
7	Serino	630	Campania	18
8	Amalfi	15	Campania	12
9	S. Severino Lucano	880	Basilicata	14
10	Viggianello	560	Basilicata	8
11	Taverna	670	Calabria	18
12	Stilo	520	Calabria	10

MATERIALS AND METHODS

We collected 149 specimens of *Salamandrina terdigitata* from 12 populations covering almost the entire range of the species (Fig. 1). The geographical origin of the samples and sample sizes are presented in Table 1. Each specimen was anaesthetized in the field with 3-aminobenzoic acid ethyl ester (MS222) and tail-clipped (about 2 cm) before being released in the same place. Tail samples were transported to the laboratory in liquid nitrogen containers and stored at –80 °C until further analyses. In order to adjust technical procedures and score liver-active enzymes, five specimens from each sampling site were euthanased with an excess of MS222. Samples of skeletal muscle and liver were then obtained and stored at -80 °C.

Tissues from each specimen were crushed in 0.1 ml of distilled water and adsorbed onto chromatography paper labels. Horizontal electrophoresis was carried out onto 10% starch gels. We studied electrophoretically 20 enzymes encoded by 29 presumptive loci (see Table 2 for description of systems and electrophoretic conditions). Isozymes were numbered in order of decreasing mobility from the most anodal one (*Ldh-1* and *Ldh-2* correspond to *Ldh-A* and *Ldh-B* respectively). Alleles at each locus were designated by their mobility (in mm, standardized conditions) relative to the most common one (100) in the reference population (Taverna, Calabria).

TABLE 2. Enzymes studied in *S. terdigitata*, their commission number (EC), encoding loci, buffer systems and tissues used in electrophoresis (M = skeletal muscle, L = liver). Buffer systems: 1) Discontinous Tris/Citrate pH 8.7 (Poulik, 1957); 2) Continous Tris/Citrate pH 8.0 (Selander *et al.*, 1971); 3) Tris/Versene/Borate pH 8.0 (Brewer & Sing, 1970); 4) Tris/Maleate pH 7.4 (Brewer & Sing, 1970); 5) Phosphate-Cytrate pH 6.3 (Harris, 1966); 6) Histidine/Citrate pH 7 (Cheliak & Pitel, 1984); 7) Lithium-borate pH 8.3 (Soltis *et al.*, 1983).

Enzyme	EC	Encoding loci	Buffer systems	Tissue
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3pdh*	5	M
Lactate dehydrogenase	1.1.1.27	*Ldh-1*	4	M
		Ldh-2	4	M
Malate dehydrogenase	1.1.1.37	*Mdh-1*	5	M
Malate dehydrogenase ($NADP^+$)	1.1.1.40	*Mdhp-1*	2,5	M, L
		Mdhp-2	2,5	M, L
Isocitrate dehydrogenase	1.1.1.42	*Icdh-1*	6	M
		Icdh-2	6	M
6-phosphogluconate dehydrogenase	1.1.1.44	*6Pgdh*	5	M
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6pdh*	1	M
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*Gapdh*	6	M
Superoxide dismutase	1.15.1.1	*Sod-1*	3	M
Aspartate transaminase	2.6.1.1	*Aat-1*	5	L, M
		Aat-2	5	L, M
Alanine transaminase	2.6.1.2	*Alat*	2, 7	L
Creatine kinase	2.7.3.2	*Ck*	2	L
Adenylate kinase	2.7.4.3	*Adk*	2	L
L-leucylglycylglycine peptidase	3.4.13	*Pep-B1*	7	M, L
		Pep-B2	7	M, L
L-phenylalanyl-L-proline peptidase	3.4.13.9	*Pep-D1*	2, 7	M, L
		Pep-D2	2, 7	M, L
Carbonic anhydrase	4.2.1.1	*Ca-2*	3	M
Triose-phosphate isomerase	5.3.1.1	*Tpi*	2	L
Mannose phosphate isomerase	5.3.1.8	*Mpi*	3	M
Glucose phosphate isomerase	5.3.1.9	*Gpi*	3	M
Phosphoglucomutase	5.4.2.2	*Pgm-1*	4	M
		Pgm-2	4	M
		Pgm-3	4	M
		Pgm-4	4	M

TABLE 3. Allele frequencies and estimates of genetic variability at 16 polymorphic loci for the 12 populations sampled. P_{95}=percentage of polymorphic loci with the most common allele not exceeding 95%. A=mean number of alleles per locus. H_e= average expected heterozygosity assuming Hardy-Weinberg equilibrium. H_o=average observed heterozygosity. Standard errors in parentheses.

		Population											
Locus		1	2	3	4	5	6	7	8	9	10	11	12
Ldh1	100	0.79	0.83	0.90	0.95	0.93	1.00	0.17	-	0.23	0.06	0.97	0.80
	110	0.21	0.17	0.10	0.05	0.07	-	-	-	-	-	-	-
	112	-	-	-	-	-	-	0.83	1.00	0.77	0.94	0.03	0.20
Mdhp1	100	-	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00
	102	0.17	-	-	-	-	-	-	-	-	-	-	-
	104	0.83	1.00	1.00	1.00	0.36	0.25	-	-	-	-	-	-
	108	-	-	-	-	0.64	0.75	-	-	-	-	-	-
Mdhp2	100	-	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00
	112	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-	-	-	-
Icdh2	88	1.00	1.00	1.00	1.00	1.00	1.00	-	-	0.27	0.42	0.50	0.44
	100	-	-	-	-	-	-	1.00	1.00	0.73	0.58	0.50	0.56
Gapdh	100	-	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00
	110	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-	-	-	-
Aatl	100	-	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00
	120	0.50	1.00	0.88	1.00	1.00	0.25	-	-	-	-	-	-
	130	0.28	-	0.12	-	-	0.75	-	-	-	-	-	-
	140	0.22	-	-	-	-	-	-	-	-	-	-	-
Alat	70	0.10	-	-	-	-	-	-	-	-	-	-	-
	80	0.90	1.00	1.00	1.00	1.00	1.00	-	-	-	-	-	-
	100	-	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00
PepB1	95	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-	-	-	-
	100	-	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00
PepD2	100	-	-	-	-	-	-	1.00	1.00	0.94	0.88	1.00	1.00
	105	0.17	-	-	0.06	-	-	-	-	-	-	-	-
	110	0.83	1.00	0.88	0.56	0.50	0.85	-	-	-	-	-	-
	115	-	-	-	-	-	-	-	-	0.06	0.12	-	-
	120	-	-	0.12	0.38	0.50	0.15	-	-	-	-	-	-
Ca2	90	-	-	-	-	-	-	-	-	-	0.19	0.25	0.40
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.81	0.75	0.60
Tpi	100	-	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00
	105	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-	-	-	-
Mpi	95	0.70	0.69	0.60	0.70	0.83	0.85	-	-	-	-	-	-
	100	0.30	0.31	0.40	0.30	0.17	0.15	1.00	1.00	1.00	1.00	1.00	0.95
	106	-	-	-	-	-	-	-	-	-	-	-	0.05
Gpi	95	0.15	0.44	0.30	0.23	0.29	0.25	-	-	-	-	-	-
	100	0.85	0.56	0.70	0.77	0.71	0.75	1.00	1.00	1.00	1.00	1.00	1.00
Pgm2	100	-	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00
	103	-	-	-	-	0.07	0.30	-	-	-	-	-	-
	106	1.00	1.00	1.00	1.00	0.93	0.70	-	-	-	-	-	-
Pgm3	90	-	-	-	-	-	-	-	-	-	-	0.03	-
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	1.00
	108	-	-	-	-	-	-	-	-	-	-	0.03	-
Pgm4	90	-	-	-	-	-	-	-	-	-	-	0.04	-
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.92	1.00	0.89	0.95
	106	-	-	-	-	-	-	-	-	0.08	-	0.07	0.05
	P_{95}	24.1	10.3	17.2	10.3	20.7	20.7	3.4	0.0	13.8	13.8	13.8	17.2
	A	1.3	1.1	1.2	1.2	1.2	1.2	1.0	1.0	1.1	1.1	1.2	1.2
		(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.0)	(0.0)	(0.1)	(0.1)	(0.1)	(0.1)
	H_e	0.08	0.04	0.06	0.05	0.07	0.08	0.01	0.00	0.04	0.04	0.04	0.06
		(0.03)	(0.02)	(0.03)	(0.03)	(0.03)	(0.03)	(0.01)	(0.00)	(0.02)	(0.02)	(0.02)	(0.03)
	H_o	0.09	0.04	0.06	0.03	0.07	0.07	0.01	0.00	0.04	0.03	0.03	0.06
		(0.03)	(0.02)	(0.03)	(0.02)	(0.03)	(0.03)	(0.01)	(0.00)	(0.02)	(0.02)	(0.01)	(0.03)

Allele frequencies and estimates of genetic variability – mean observed and expected heterozygosity (H_o and H_e), percentage of polymorphic loci (P) and average number of alleles per locus – were calculated for each population using the software BIOSYS-2 (Swofford & Selander, 1999). Exact significance tests for Hardy-Weinberg equilibrium (HW) were conducted for each locus and sample, then the Bonferroni correction for multiple tests was applied (Rice, 1989).

Genetic distances between populations were calculated with Nei's (1972) standard genetic distance matrix, which was then used to build an UPGMA phenogram. We ran 1000 bootstrap pseudoreplicates over loci to test the reliability of the UPGMA phenogram with the BOOTDIST option in BIOSYS-2. The consensus UPGMA was then obtained using the subroutines NEIGHBOR and CONSENSE in the software PHYLIP 3.5c (Felsenstein, 1993). The cophenetic correlation coefficient, which measures the correlation between distance values calculated during tree building and the observed distance, was also computed. In addition, a hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was carried out in order to partition total genetic variance into covariance components due to differences within populations, among populations within groups and between groups, using ARLEQUIN 2.000 (Schneider *et al.*, 1999).

RESULTS

Thirteen out of 29 loci analysed (*G3pdh, Ldh2, Mdh1, Icdh1, 6Pgdh, G6pdh, Sod1, Aat2, Ck, Adk, PepB2, PepD1,* and *Pgm1*) were monomorphic in all populations surveyed. Allele frequencies at the sixteen polymorphic loci are presented in Table 3. None of the tests for HW equilibrium was significant after Bonferroni correction. Based on allele frequencies at polymorphic loci, the samples can be classified into two well-differentiated groups, one comprising samples from the Tusco-Emilian Apennine to southern Latium (samples 1–6), and the other including those from central Campania to southern Calabria (samples 7–12). This subdivision is reflected by the two main clusters in the UPGMA phenogram showed in Fig. 2 (cophenetic correlation coefficient CCC=0.995). Nine loci (*Mdhp1, Mdhp2, Gapdh, Aat1, Alat, PepB1, PepD2, Tpi, Pgm2*) were fully diagnostic between both groups of populations, while at the other four loci (*Ldh1, Icdh2, Mpi, Gpi*) distinct alleles were found at moderate to high frequencies in only one of these groups (see Table 3). Genetic distances – D_{Nei} – between the two population groups ranged from 0.41 to 0.52, with an average value of 0.47 (SD=0.03), whereas within each of the two groups D_{Nei} varied from 0.00 to 0.05, with an average value of 0.02 (SD=0.02). Within the southern group, populations 11 and 12 (Calabria) were the most differentiated, with high bootstrap support (88%) in the UPGMA analysis and presenting an average genetic distance D_{Nei}=0.03 (SD=0.01) with respect to the other

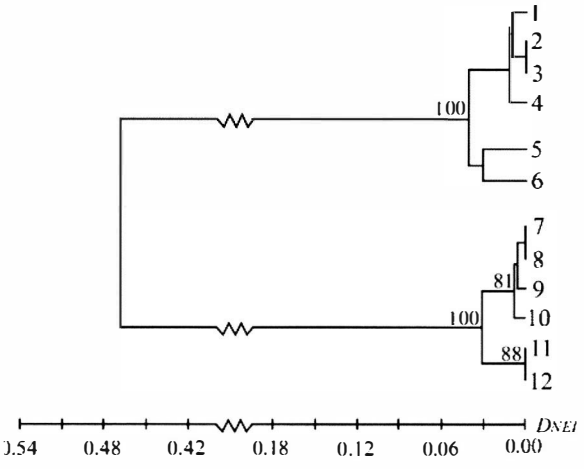

FIG. 2. UPGMA tree showing genetic relationships among the 12 populations of *S. terdigitata*, based on Nei's (1972) standard genetic distance values. Bootstrap values >70% over 1000 pseudoreplicates are indicated. Populations are numbered as in Table 1.

southern samples. When populations were grouped into the two main groups identified by the UPGMA analysis, the results of the AMOVA analysis (Table 4) indicated that up to 91% of the total genetic variability found in our dataset can be attributed to differences among groups.

Estimates of genetic diversity are presented in Table 3. Expected heterozygosity varied from 0.00 to 0.08, with the highest values in populations from the central group (populations 1, 5 and 6). Within the southern group, the population from Amalfi completely lacks genetic diversity, and that from Serino was polymorphic at only one locus (*Ldh1*), with H_e=0.01. Other measures of genetic variability (observed heterozygosity, percentage of polymorphic loci and average number of alleles per locus) exhibited the same geographical pattern as H_e.

DISCUSSION

We observed a particularly significant pattern of genetic differentiation among the studied populations, mainly due to the existence of two well-defined clusters of closely related samples, one comprising populations from central Italy (samples 1 to 6), and the other those from southern Italy (samples 7 to 12). The pattern of genetic divergence between the two groups (leading to an average D_{Nei} of 0.47) was found to be unexpectedly high considering the restricted distribution of this species (it is an Apennine endemic), as well as its apparent morphological homogeneity. The level of divergence observed resembles that found between several congeneric species of salamanders (see, for example, Macgregor *et al.*, 1990; Lanza *et al.*, 1995; Nascetti *et al.*, 1996). When also considering the existence of nine fully diagnostic loci between the two groups, as well as the full concordance with the mitochondrial DNA data (Nascetti *et al.*, 2005), the observed overall genetic pattern indicates that the two well-differentiated

TABLE 4. AMOVA results for *S. terdigitata* data obtained using ARLEQUIN 2.000 (Schneider *et al.*, 1999). Groups were defined as the two main clusters identified by the UPGMA cluster analysis (Fig. 2).

Source of variation	Percentage of variation	Fixation indices	*P*-values
Among groups	91.38	F_{ct} = 0.914	<0.01
Among populations within groups	1.47	F_{sc} = 0.171	<0.01
Within populations	7.14	F_{st} = 0.929	<0.01

evolutionary lineages found within the spectacled salamander might actually represent two distinct species (see below).

Substantial genetic homogeneity was found within both population groups, notwithstanding the fact that within the southern group the populations from Calabria showed some distinctiveness according to the UPGMA analysis. For several populations, the levels of genetic diversity resemble the average values reported by Nevo & Beiles (1991) for representatives of the family Salamandridae (H_e=0.058; P=24.0%). However, the sample from Amalfi lacks genetic diversity at the studied loci, and the sample from Serino showed almost no genetic diversity. This could be a result of either human-driven depletion such as that caused by habitat destruction, population isolation, etc. (e.g. Haig, 1998), or the marginal position of these populations within the species range (Ledig, 1986), or from a combination of both factors. At this time we are unable to distinguish between these possible causes, even if the poor conservation status and fragmentation of the habitats in the underlying geographic area are likely to have played some role.

NOMENCLATURE DESIGNATION

As mentioned by Lanza (1988), the spectacled salamander was originally described by Lacépède (1788) with the name *Salamandra ter-digitata* on the basis of a single specimen collected by M. le Comte de Milly from Vesuvius. Fifteen years later, the species was also described (under the name *Salamandra tridactyla*) by Daudin (1803), who based his description on a specimen collected by De Nesle in the same locality. Later, another description was published by Savi (1821), who described the new species *Salamandra perspicillata* from cool and shady sites of Tuscan Apennine and particularly Mugello. He believed that he had found a new salamander species, because Lacépède's salamander was described as having four toes on the hind feet, but only three toes on the front feet. Later studies indicated that the two salamanders belonged to the same species, the original description by Lacépède being based on a poorly preserved specimen. Following the principle of priority of the International Code of Zoological Nomenclature, the species was given the name chosen by Lacépède, changed into *terdigitata* by removing the hyphen. Finally, Fitzinger (1826) proposed that the species belonged to a distinct monotypic genus, which he named *Salamandrina*. Other names have subsequently been proposed for the species, but they were all later synonymized (e.g. *Salamandra imperati* Costa, 1828; *Salamandra savi* Cuvier, 1829).

A recent decision of the International Commission on Zoological Nomenclature (Opinion 2104, based on the application of Savage, 2003) states that the work by Lacépède (1788) is no longer available as a source. As also pointed out by Savage (2003), this decision does not affect the name for *Salamandrina terdigitata* and other Latinized vernacular names from the Lacépède's (1788) work, because they "...were given proper binomials based on Lacépède's names in Bonnaterre's (1789–1790) binomial work". Nevertheless, this decision affect the authorship and year of the species' description. With the rejection of Lacepede's work, the correct author for *Salamandrina terdigitata* is now Bonnaterre (1789).

According to the principle of chronological priority and the geographic origin of our samples, we suggest that the name *Salamandrina perspicillata* (Savi, 1821) should be used for the species from central Italy, while the name *Salamandrina terdigitata* (Bonnaterre, 1789) should be retained for the southern species. However, this new nomenclatural arrangement will need to be further discussed, depending on the identity of the type specimens being confirmed. The analyses of type specimens will follow the completion of morphological analyses, now in progress, to identify potential morphological differences of diagnostic value between the two species. At present the two species can be diagnosed based on their genetic divergence, as assessed at diagnostic allozyme loci (*Mdhp1, Mdhp2, Gapdh, Aat1, Alat, PepB1, PepD2, Tpi, Pgm2*) and mitochondrial DNA sequences (Nascetti *et al.*, 2005). Moreover, the geographic origin of specimens is also of diagnostic value, although the species assignment of populations from the central portion of the Volturno river drainage basin still needs to be assessed (see Fig. 1).

CONCLUSIONS

The main outcome of this study has been the recognition of two distinct species within the Italian endemic *Salamandrina terdigitata*. Future efforts will be focused on two main fields: morphological variation, and genetic analyses of intermediate populations.

In many amphibians, previously undescribed morphological variation has often been assessed after species recognition in genetic studies (e.g. Nascetti *et al.*, 1988; Nascetti *et al.*, 1995). Since no studies to date have investigated morphological variation within the spectacled salamander, future studies will attempt to identify potential differences of diagnostic value between *S. terdigitata* and *S. perspicillata* at the level of chromatic, morphological and osteological characters.

However, preliminary observations suggest that some chromatic differences do exist between the species. The ventral surface of the tail exhibits a bright red in *S. terdigitata*, whereas it looks reddish to brownish-orange in *S. perspicillata*. In addition, the demarcation between the dorsal and ventral coloration of the tail is sharper in *S. terdigitata* than in *S. perspicillata*.

With respect to genetic studies, further sampling will be focused on potential contact zones, such as mid-altitude areas of the Volturno river drainage basin (see Fig. 1). The main objectives of the study of these populations will be: (1) to define the reciprocal distribution of the two species (i.e. if allopatric or parapatric); (2) to ascertain whether a contact zone does exist and, if so, to delimit it and to address what kind of genetic and ecological interactions are occurring between both species in this area.

Finally, the split of the former *S. terdigitata* into two distinct species with restricted geographical distributions strongly suggests the necessity for a careful evaluation of their conservation status, as well as the relevance of including them in the main international lists of threatened species, such as the IUCN Red List.

NOTE ADDED IN PROOF

Recently, a further paper has also been published describing mitochondrial DNA variation within the spectacled salamander (Mattoccia *et al.*, 2005). Since the data presented within this paper substantially confirms the previous findings of Nascetti *et al.* (2005), they will not be further discussed here.

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REFERENCES

Bonnaterre, P.-J. A. (1789). *Erpétologie*. xxviii, 71 pp. Paris: Panckoucke Lib.

Borkin, L. J. (1999). Distribution of amphibians in North Africa, Europe, Western Asia, and the former Soviet Union. In: *Patterns of Distribution of Amphibians: A Global Perspective*, 329–420. Duellmann, W. E. (Ed.). Baltimore: The John Hopkins University Press.

Brewer, G. J. & Sing, C. F. (1970). *An Introduction to Isozyme Techniques*. New York & London: Academic Press.

Cheliak, W. M. & Pitel, J. A. (1984). *Techniques for Starch Gel Electrophoresis of Enzymes from Forest Tree Species*. Petawawa National Forestry Institute, Information Report P1-X-42. Chalk River, Ontario: Canadian Forestry Service, Agriculture.

Cherty, L. M., Case, S. M. & Wilson, A. C. (1978). Frog perspective on the morphological difference between humans and chimpanzees. *Science* **200**, 209–211.

Corsetti, L. & Angelini, C. (2000). *Salamandrina terdigitata*. In *Anfibi e Rettili del Lazio*, 38–39. Bologna, M. A., Capula, M. & Carpaneto, G. M. (Eds.). Roma: Palombi editore.

Daudin, F. M. (1803). *Histoire Naturelle, Générale et Particulière des Reptiles*, vol. VIII. Paris: de l'Imprimerie de F. Dufart.

Duellman, W. E. (1993). *Amphibian Species of the World: Additions and Corrections*. Lawrence, KS: University of Kansas Printing Service.

Excoffier, L., Smouse, P. E. & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491.

Felsenstein, J. (1993). *PHYLIP (Phylogenetic Inference Package) and Manual, version 3.5c*. Seattle: Department of Genetics, University of Washington.

Fitzinger, L. J. (1826). *Neue Classification der Reptilien nach ihren natürlichen Verwandschaften nebst einer Verwandtschaften-Tafel und einem Verzeichnisse der Reptilien-Sammlung des K. K. Zoologischen Museums zu Wien*. Vienna: J. G. Huebner.

Frost, D. R. (2002). *Amphibian Species of the World: An Online Reference (V2.21)*. Electronic database available at http://research.amnh.org/herpetology/amphibia /index.html.

Haig, S. M. (1998). Molecular contributions to conservation. *Ecology* **79**, 413-425.

Hanken, J. (1999). Why are there so many new amphibian species when amphibians are declining? *Trends in Ecology and Evolution* **14**, 7-8.

Harris, H. (1966). Enzyme polymorphism in man. *Proceeding of the Royal Society London, Series B* **164**, 298–310.

Hass, C. A., Dunski, J. F. & Maxson, L. R. (1995). Divergent lineages within the *Bufo margaritifera* complex (Amphibia: Anura; Bufonidae) revealed by albumin immunology. *Biotropica* **27**, 238–249.

Hudson, R. D. & Coyne, J. A. (2002). Mathematical consequences of the genealogical species concept. *Evolution* **56**, 1557–1565.

Lacépède, B. G. E. (1788). *Histoire naturelle des quadrupèdes ovipares et des serpens*, Vol. 1. Paris: Imprimerie du Roi (Hôtel de Thou).

Lanza, B. (1983). Anfibi e Rettili. *Guide per il Riconoscimento delle Specie Animali delle Acque Interne Italiane* **27**, 64–70.

Lanza, B. (1988). *Salamandrina terdigitata* (Lacépède, 1788): emblem of the Unione Zoologica Italiana. *Bollettino di Zoologia* **55**, 1–4.

Lanza, B., Caputo, V., Nascetti, G. & Bullini, L. (1995). Morphologic and genetic studies of the European plethodontid salamanders: taxonomic inferences (genus *Hydromantes*). *Monografie* **XVI**, Museo Reg. Sc. Nat., Torino.

Ledig, F. T. (1986). Heterozygosity, heterosis, and fitness in outbreeding plants. In *Conservation Biology: The Science of Scarcity and Diversity*, 77–104. Soulé, M. E. (Ed.). Sunderland, MA: Sinauer Associates.

Macgregor, H. C., Sessions, S. K. & Arntzen, J. W. (1990). An integrative analysis of phylogenetic relationships among newts of the genus *Triturus* (family Salamandridae), using comparative biochemistry, cytogenetics and reproductive interactions. *Journal of Evolutionary Biology* **3**, 329–373.

Mattoccia, M., Romano, A. & Sbordoni, V. (2005). Mitochondrial DNA sequence analysis of the spectacled salamander, *Salamandrina terdigitata* (Urodela: Salamandridae), supports the existence of two distinct species. *Zootaxa* **995**, 1-19.

Mazzotti, S., Caramori, G. & Barbieri, C. (1999). Atlante degli anfibi e dei rettili dell'Emilia-Romagna (aggiornamento 1993/1997). *Quaderni della Stazione Ecologica del Museo Civico di Storia Naturale di Ferrara* **12**, 1–121.

Mertens, R. & Wermuth, H. (1960). *Die Amphibien und Reptilien Europas* (Dritte Liste, nach dem Stand vom 1. Januar 1960). Frankfurt/M: Kramer.

Nascetti, G., Androne, F., Capula, M. & Bullini, L. (1988). A new *Salamandra* species from southwestern Alps (Amphibia, Urodela, Salamandridae). *Bollettino del Museo Regionale di Scienza Naturali di Torino* **6**, 617-638.

Nascetti, G., Lanza, B. & Bullini, L. (1995). Genetic data support the specific status of the Italian treefrog (Amphibia: Anura: Hylidae). *Amphibia-Reptilia* **16**, 215–227.

Nascetti, G., Cimmaruta, R., Lanza, B. & Bullini, L. (1996). Molecular taxonomy of European plethodontid salamanders (Genus *Hydromantes*). *Journal of Herpetology* **30**, 161–183.

Nascetti, G., Zangari, F. & Canestrelli, D. (2005). The spectacled salamanders, *Salamandrina terdigitata* Lacépède, 1788 and *S. perspicillata* Savi, 1821: 1) genetic differentiation and evolutionary history. *Rendiconti Lincei: Scienze Fisiche e Naturali* **16**, 159–169.

Nei, M. (1972). Genetic distance between populations. *The American Naturalist* **106**, 283–292.

Nevo, E. & Beiles, A. (1991). Genetic diversity and ecological heterogeneity in amphibian evolution. *Copeia* **1991**, 565–592.

Poulik, M. D. (1957). Starch gel electrophoresis in a discontinuous system of buffers. *Nature* **180**, 1477.

Rice, W. R. (1989). Analysing tables of statistical tests. *Evolution* **43**, 223–225.

Richards, C. M. & Moore, W. S. (1996). A phylogeny for the African treefrog family Hyperoliidae based on mitochondrial DNA. *Molecular Phylogenetics and Evolution* **5**, 522–532.

Savage, J. M. (2003). Case 3226. Lacépède, B. G. E. de la V., 1788, Histoire Naturelle des Quadrupèdes Ovipares: proposed rejection as a non-binominal work. *Bulletin of Zoological Nomenclature* **60**, 138–140.

Savi, P. (1821). Descrizione (inedita) di una nuova specie di Salamandra terrestre, *Salamandra perspicillata* Nob. *Biblioteca italiana (Giornale di Letteratura, Scienze ed Arti)* **22**, 228–230.

Schneider, S., Roessli, D. & Excoffier, L. (1999). *ARLEQUIN ver 2.0: Software for Population Genetic Data Analysis*. Geneva, Switzerland: University of Geneva Genetics and Biometry Laboratory.

Selander, R. K., Smith, M. H., Yang, S. Y., Johnson, W. E. & Gentry, J. B. (1971). Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse. University of Texas Publications **7103**, 49–90.

Soltis, D. E., Haufler, C. H., Darrow, D. C. & Gastony, G. J. (1983). Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American Fern Journal* **73**, 9–27.

Swofford, D. L. & Selander, R. B. (1999). *BIOSYS-2: A Computer Program for the Analysis of Allelic Variation in Population Genetics and Biochemical Systematics (Release 2.0)*. Urbana-Champaign, IL: University of Illinois.

Titus, T. A. & Larson, A. (1995). A molecular phylogenetic perspective on the evolutionary radiation of the salamander family Salamandridae. *Systematic Biology* **44**, 125–151.

Veith, M. (1996). Molecular markers and species delimitation: examples from the European batrachofauna. *Amphibia–Reptilia* **17**, 303–314.

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SHORT NOTES

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FIRST DESCRIPTION OF THE ACOUSTIC REPERTOIRE OF *RANA ITALICA* (ANURA, RANIDAE)

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Vocalizations of *Rana italica* from six adult males were obtained from Torrente Gentile, a small brook located in the Torrente San Siro basin, Santa Margherita Ligure (Northern Italy). All signals were propagated from the bottom of shallow pools and were inaudible in air. Two common and one rare call type were documented. All calls were short, low amplitude signals. The most common call is frequency modulated, harmonically rich and the other two are not. None exceed 0.26 s. Our study confirms that males call underwater. This is consistent with the conclusion that it has an underwater mate recognition system.

Key words: frog, reproductive behaviour, underwater propagation, vocalizations

The Italian stream frog is widespread within the Italian Peninsula , and prefers small streams within the wet, broad-leaf forests of the Apennines from central Liguria to Calabria (Zuffi, 1997; Picariello *et al*., 2006). Despite its abundance and distribution, information about the ecology of *R. italica* is scanty (Guarino *et al.*, 1993), and no detailed study of its breeding ecology has been done to date. Both males and females remain near stream pools for most of the year, using rocks on the bottom of deeper pools with permanent water as refuges against predators, as well as for mating and egg laying (Lanza, 1983).

Breeding occurs from February to May and peak breeding activities occur in March (Guarino *et al*., 1993). Males attract females by emitting weak, frequently repeated calls, which are uttered underwater, and are generally not audible in air unless frogs are calling just below the water surface. This is probably the main reason why no description of the vocal repertoire of this species has been published to date. The purpose of this study is therefore to characterize the vocalizations of *R. italica*.

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We recorded male calls at Torrente Gentile, a small brook located in the Torrente San Siro basin, in Santa Margherita Ligure Municipality (Northern Italy, 44°20'N, 9°12'E). Vocal signals of frogs were recorded on 22 March 2003 between 22:00 and 24:00 hrs in two adjacent pools (each one approximately 6 m^2; elevation 150 m a.s.l.). The first pool was about 80 cm deep, and the second about 1 m. The water temperature was 9.6 C° in both pools, and did not vary during the recording time. Recordings were obtained using a omnidirectional MPC piezo hydrophone (Dolphinear, Arretec, frequency range 7 – 22,000 Hz) and a Sony TC-D5 cassette recorder. All records were made at 9.5 cm/s tape speed. During recording sessions it was not possible to hear the frogs calling from the border of the pools without the hydrophone, despite the fact that the background noise caused by the flow of water was quite low. We obtained a total of 25 minutes of recordings that included 176 vocalizations from a total of six different males (average= 29±8 SE for individual, range: 11- 66). Call sites varied between 30 and 90 cm deep. Calling males were unambiguously identified by their position within the pools (four frogs called from the first pool and two from the second one). The distance between a calling male and the hydrophone was small, varying from 30-50 cm. Vocalizations obtained from each male were analysed using Cool Edit Pro v. 2.0 (Syntrillium). The best resolution was achieved analysing vocalizations in the 0-11 KHz frequency range, using a sampling rate of 22,050 sample/s; band-width 28 Hz; a frequency resolution 10 Hz and time resolution 23 ms. We visually examined and compared spectrograms in order to identify the different call types. For each call we measured the following four variables (when applicable): call duration (ms), time to peak amplitude (ms), dominant frequency (Hz) and fundamental frequency (Hz). Unless otherwise stated, values reported are means ± SE.

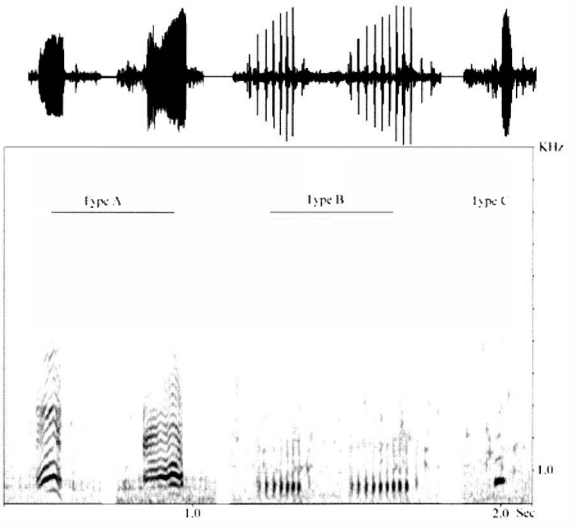

FIG. 1. Sonagrams and waveforms of call types of *Rana italica*.

TABLE 1. Summary of spectrographic features of call types of *Rana italica*.

	Call types		
	A	B	C
SAMPLE SIZE			
n of individuals	6	4	6
n of calls	135	28	13
n of calls per individual	22.5±7.3 (9-58)	7±2.4 (2-13)	2.2±0.5 (1-4)
CALL FEATURES			
Duration (ms)	152±8.9 (135-162)	173±10.7 (149-260)	81±4.2 (68-87)
Time of the peak amplitude (ms)	111±8.7 (89-120)	130±10.2 (93-212)	57±5.1 (49-66)
Fundamental frequency (Hz)	297±23.9 (225-368)	–	731±16.1 (646-754)
Dominant Frequency (Hz)	1059±112 (765-1202)	611±18.8 (553-723)	731±16.1 (646-754)

Calls could be unambiguously assigned to three different call types based on the shape of their spectrogram and audibly by observers. All the individuals analysed shared the vocal repertoire described below.

The sonagrams and oscillograms are provided in Fig. 1. The description of the several call types are here provided.

Call type A is a harmonically rich, frequency modulated call that sounded similar to a short scream or a "squack", with a mean duration of 152±8.9 ms (range 135-162, *n*=6) and a mean fundamental frequency is 297±23.9 Hz (range 225-368, *n*=6). Peak amplitude was variable but typically increased gradually to a maximum after an average of 111±8.7 ms (range 89-120, *n*=6). Dominant frequency averaged 1059 ± 112 Hz but varied widely (range 765-1202, *n*=6); corresponding generally to the fourth harmonic (Fig. 1, Table 1).

Call type B was of lower intensity, and resulted in a "grongron" like utterance somewhat similar to the call of *Rana dalmatina* (Nöllert & Nöllert, 1992). Call type B was quite different from type A, it was a pulsed sound lacking frequency modulation and composed of 5 to 15 pulses (average 7.9±0.4, *n*=4). Pulses were regularly spaced and most of the energy was concentrated at the lower frequencies. Dominant frequency averaged 611±18.8 Hz and varied from 553-723, (*n*=4). Call duration varied substantially, averaging 173±10.7 ms (range 149-260, *n*=4). The amplitude of the pulses increased gradually and a maximum was reached after 130±10.2 ms on average but varied two-fold (range 93-212 ms, *n*=4).

Call type C is a short, high-pitched note that sounded like an "uh". Average duration was 81±4.2 ms that varied much less than the other call types (68-87, *n*=6). Fundamental frequency corresponded to the dominant frequency, averaging 731±16.1 Hz (range 646-754, *n*=6). Amplitude increased to a maximum value after 58±5.1 ms (range 49-66, *n*=6). This call type showed only slight harmonic structure.

Call type A was the most frequently recorded (135 calls); by contrast, call type C was rarely recorded. Among 176 calls, type C was documented only 13 times. We decided not to capture or otherwise disturb the calling males during or after recording sessions. In addition, any external light caused the abrupt cessation of all singing, and therefore we were unable to observe any behaviour that could be associated with a given call type. However, based on other related European ranid frog species (*R. graeca*, Asimakopoulos *et al.*, 1990; Asimakopoulos, 1994; *R. latastei*, Farronato *et al.*, 2000), and the time within the breeding season, we provisionally consider call type A to represent a male advertisement call. Call type B may represent some form of territorial spacing signal, or an agonistic element. Call type C may represent a release call. Further recordings under controlled conditions that permit simultaneous recordings and visual observations should provide further information on call function.

REFERENCES

Asimakopoulos, B. (1994). The release call of the greek frog, *Rana graeca* (Boulenger, 1891). A preliminary report. *Bios* (Macedonia, Greece) **2**, 91–94.

Asimakopoulos, B., Sofianidou, T. S. & Schneider, H. (1990). Reproductive and calling behavior of the Greek Frog *Rana graeca* (Amphibia: Anura) in Greece. *Zoologischer Anzeiger* **225**, 133–143.

Farronato, I., Pesente, M., Fracasso, G. & Parlotto, L. (2000). Osservazioni sulle manifestazioni sonore di *Rana latastei* Boulenger, 1879. In *Atti 3° Convegno Faunisti Veneti*, 32–36. Bon, M. and Scarton, F., (Eds). Rovigo: Museo Civico di Storia Naturale di Venezia.

Guarino, F. M., Di Fiore, M. M., Caputo, V., Iela, L., Angelini, F. & Rastogi, R. K. (1993). Seasonal analysis of the reproductive cycle in two wild populations of *Rana italica* Dubois 1985. *Animal Biology* **2**, 25–43.

Lanza, B. (1983). *Guide per il riconoscimento delle specie animali delle acque interne italiane. 27. Anfibi, Rettili (Amphibia, Reptilia)*. Roma: AQ/1/205, CNR.

Nöllert, A. & Nöllert, C. (1992). *Die Amphibien Europas: Bestimmung, Gefährdung, Schutz*. Stuttgart: Franckh Kosmos Verlags-GmbH & Co.

Picariello, O., Guarino, F. M. & Barbieri, F. (2006). *Rana italica* Dubois, 1987. In *Atlante degli Anfibi e dei Rettili d'Italica/Atlas of Italian Amphibians and Reptiles*, 358–361. Bernini, F., Doria, G., Razzetti, E., Sindaco, E. (Eds). Firenze: Polistampa.

Zuffi, M. A. L. (1997). *Rana italica* Dubois, 1987. In *Atlas of Amphibians and Reptiles in Europe*, 144–145. Gasc, J. P., Cabela, A., Crnobrnja-Isailovic, J., Haffner, P., Lescure, J., Martens, H., Martínez Rica, J. P., Maurin, H., Oliveira, T., Sofianidou, T. S., Veith, M. and Zuiderwijk, A. (Eds). Paris: Societas Europaea Herpetologica & Muséum National d'Histoire Naturelle (IEGP/SPN).

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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.

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