Journal of Herpetology, Vol. 39, No. 3, pp. 366–372, 2005

Life-History Responses to Pathogens in Tiger Salamander (*Ambystoma tigrinum*) Larvae

MATTHEW J. PARRIS,[1,2] ANDREW STORFER,[3] JAMES P. COLLINS,[4] AND ELIZABETH W. DAVIDSON[4]

[1]*Department of Biology, University of Memphis, Memphis, Tennessee 38152, USA; E-mail: mparris@memphis.edu*
[3]*School of Biological Sciences, Washington State University, Pullman, Washington 99164-4236, USA*
[4]*School of Life Sciences, Arizona State University, Box 874501, Tempe, Arizona 85287-4501, USA*

ABSTRACT.—**We tested whether the presence of an iridovirus (*Ambystoma tigrinum* virus; ATV) could alter patterns of larval life histories in Arizona Tiger Salamanders (*Ambystoma tigrinum nebulosum*). Viral epidemics cause extreme mortality in natural populations and, thus, impose a strong selective force. We tested how exposure to ATV during larval development influences survival, growth, and frequency of cannibalism by manipulating the presence of ATV in replicated experimental tanks. ATV significantly reduced survival and larval growth. Propensity to become cannibalistic was not related to ATV exposure, suggesting that salamanders cannot facultatively respond to the presence of diseased conspecifics by reducing cannibalism. Our results demonstrate that viral pathogens may have both a direct and indirect effect on *A. tigrinum* fitness by reducing survival and growth rate.**

Ecologists increasingly recognize disease as an important factor shaping host life histories (Michalakis and Hochberg, 1994; Marcogliese and Cone, 1997; Koella et al., 1998; Day, 2003), and the consequences of pathogen-induced changes in host life histories is a critical issue in the population ecology of infectious diseases (Washburn et al., 1991; Dobson and Crawley, 1994; Kohler and Wiley, 1997; Parris et al., 2004). Manipulative studies offer a powerful approach for quantifying host-pathogen dynamics and the resultant impact of disease on host life-history evolution.

The effects of pathogens on population dynamics may be especially important for amphibians in light of recent concerns about population declines (reviewed in Daszak et al., 2003). Substantial literature suggests that pathogens have played important roles in global amphibian declines (e.g., Daszak et al., 1999; Carey, 2000; Collins et al., 2003; Kiesecker et al., 2004). Disease may affect the population dynamics of several subspecies of Tiger Salamanders, including the Arizona Tiger Salamander (*Ambystoma tigrinum nebulosum*). Jancovich et al. (1997) and Brunner et al. (2004) demonstrated that a virus (*Ambystoma tigrinum* virus; ATV) might be responsible for reoccurring epizootics. ATV and the closely related Regina ranavirus (RRV) are the first pathogenic viruses described in salamanders (Iridoviridae; Anthony and Comps, 1991; Jancovich et al., 1997; Bollinger et al., 1999). Infection is spread from sick to susceptible animals through water or by direct contact (Jancovich et al., 2001). Most animals die within 2–3 weeks of first symptoms, which include lethargy, extreme epidermal sloughing, and hemorrhaging.

Tiger Salamanders (*A. tigrinum*) are found throughout North America (Shaffer and McKnight, 1996) and are excellent models for studying effects of pathogens on life-history traits because ATV affects several subspecies (Collins et al., 2004; Storfer et al., 2004). Moreover, some subspecies of *A. tigrinum* exhibit a trophic polyphenism with two discrete larval morphs. Most hatchlings develop into a typical larval morph that feeds primarily on invertebrates, whereas some develop into cannibals (Gehlbach, 1969; Collins and Cheek, 1983). Cannibals have a broader head, large vomerine teeth, and a performance advantage relative to typicals because they can prey on conspecifics (Pedersen, 1991; Reilly et al., 1992; Loeb et al., 1994). Cannibalistic larvae also eat a wider range of other prey than typical larvae and can metamorphose earlier (an advantage in ephemeral habitats) and at larger body masses (Collins et al., 1993; Brunkow and Collins, 1996). Environmental cues influence the frequency of cannibal expression, including high intraspecific density that leads to resource competition (Maret and Collins, 1997). Disease provides a cost to cannibalism, and field studies show that there is an inverse correlation between frequency of disease and frequency of cannibalism in Tiger Salamanders throughout Arizona (Pfennig et al., 1991, 1994; Collins et al., 2003). However, it is unknown whether salamanders exhibit phenotypic plasticity, and thereby adjust cannibalism rates, in response to disease.

Our work was aimed at experimentally testing the role of a viral pathogen in an amphibian host-viral pathogen system and determining whether

[2] Corresponding Author.

disease can alter patterns of variation in life-history traits and affect host fitness. We also tested whether salamanders can facultatively alter the expression of the cannibalistic morph according to the infection status of conspecifics.

Materials and Methods

Source Populations and Breeding Design.—We selected salamanders from the White Mountains of east-central Arizona (33°N, 109°W) because of field data documenting occurrences of ATV epidemics and cannibalism in the region (Pfennig et al., 1991, 1994; Brunner et al., 2004). The White Mountains consist of middle- to high-elevation subalpine grasslands and coniferous forest habitats. Salamanders use a variety of aquatic environments including small lakes, ephemeral marshes, and stock tanks (Collins, 1981). Five *A. t. nebulosum* sibships were obtained from laboratory matings between animals collected from three populations in the White Mountains. Oviposition occurred from 18 April to 1 May 2001, and larvae were combined in the following proportions determined by clutch size: Lower Cottonwood Tank (two clutches) = 64%; Wildcat Point Tank (two clutches) = 26%; South Tank (one clutch) = 10%. Clutches contained approximately 100–500 larvae each, which is within the range of clutch sizes from natural populations of *A. tigrinum* (Gehlbach, 1969; Rose and Armentrout, 1976). Sibships were combined to distribute evenly population, dam, and sire effects among all treatments. After hatching, larvae were held in plastic containers (114 liters) at an approximate density of 0.11 larvae/liter, fed brine shrimp (*Artemia* sp.) ad libidum, and reared on a 12:12 L:D h photoperiod.

Experimental Design and Procedures.—We raised larvae in eight polyethylene experimental tanks (1.83 m diameter) positioned in an array at the University of Memphis Edward J. Meeman Biological Field Station (Shelby County, Tennessee; 35°22′N, 90°1′W). Tanks were exposed to natural, seasonal changes in air temperature and photoperiod. Although relatively small, experimental tanks were within the size range of small ephemeral habitats in Arizona (MJP, pers. obs.). We prepared tanks in mid-May 2001 by filling them with tap water to a depth of 30 cm (750 liters), adding 1.0 kg of air-dried leaf litter collected from nearby deciduous forests, and inoculating them seven times with 500 mL aliquots of a concentrated plankton suspension collected from several nearby natural ponds. Three adult snails (Lymnaeidae) were added to each tank to graze algae on tank surfaces. These initiation procedures established complex aquatic environments with self-maintaining food webs of algae and plankton for developing salamander larvae (Parris, 1999). No water was added during the experiment, because rainfall compensated for evaporative water loss. Securely fastened and weighted lids (fiberglass screen, 1 mm mesh) were attached to each tank and secured by attaching a tight elastic cord to provide shading and prevent colonization by predators and competitors. The relatively low water level (30 cm) minimized the probability of both pathogen and animal escape, as larvae of *A. tigrinum* and metamorphs are unable to climb the interior surfaces of experimental tanks (MJP, pers. obs.). All safety precautions were sufficient, and no further permits were necessary for this research (sensu Parris and Cornelius, 2004). We allowed tanks to condition undisturbed for 12 days before adding salamander larvae.

Treatments consisted of larvae of *A. t. nebulosum* reared in presence or absence of ATV. Larvae in all treatments were reared at an initial density of 120 larvae/tank (0.16 larvae/liter), which is within the range of natural population densities and comparable to densities used in previous experiments to induce cannibals (Collins and Cheek, 1983; Pfennig et al., 1994). The two treatment combinations were replicated either three (pathogen-free) or five (ATV-exposed) times and randomly assigned to the eight tanks. On 26 May, we haphazardly selected larvae and added them to the tanks. A sample of 10 larvae from each treatment prior to addition to the tanks indicated no significant size difference between larvae assigned to pathogen-free and ATV-exposed tanks (mean snout–vent length, SVL = 20.9 and 22.0 mm, respectively; $t_{18} = 1.23$; $P = 0.317$). Larvae were acclimated 10 days before applying virus treatments.

On 5 June, we placed two ATV-infected *A. t. nebulosum* larvae in a buoyant mesh cage in each pathogen treatment tank. Animals had been exposed 14 days earlier in the laboratory to water baths containing infectious viral titers (10^{-3} plaque-forming units; Jancovich et al., 1997). Infections of ATV were confirmed by inoculating a sample from each larva on a fish cell line (EPC; *Epithelioma papilloma cyprini*) using standard cell culture techniques (for details, see Jancovich et al., 1997). We passed each sample twice through cell culture and considered samples that exhibited a cytopathic effect on EPC cells as virus-positive. Infected animals were placed into cages ($25 \times 20 \times 12$ cm) constructed of untreated lumber with flexible plastic netting securely stapled on two surfaces, and cages were made buoyant by attaching tubular PVC floats at two ends. Because ATV can be transmitted through water in natural populations of *A. tigrinum* (Jancovich et al., 2001), naïve experimental larvae in our experiment could be affected by contact with water from the infected animals in the cages. Two noninfected larvae of *A. t. nebulosum*

TABLE 1. Summary of univariate analyses of variance for survival, snout–vent length (SVL) at day 75, and proportion of survivors becoming cannibalistic for uninfected and ATV-infected larvae of *Ambystoma tigrinum nebulosum* reared in experimental tanks.

Response	Source	df	MS	*F*	*P*
Survival	Infection Status	1	0.1206	6.61	0.0422
	Error	6	0.0182		
SVL	Infection Status	1	0.0099	13.88	0.0098
	Error	6	0.0007		
Cannibalism	Infection Status	1	0.0006	0.52	0.4986
	Error	6	0.0011		

were placed in control tank cages. Caged larvae did not differ significantly in SVL between pathogen-free (mean ± 1 SE = 70.7 ± 3.4 mm) and viral (73.5 ± 2.9 mm) treatments ($t_{14} = -1.01$; $P = 0.670$). All infected, caged larvae died within 12 days of introduction into experimental tanks, at which point they were removed. On day 12, larvae from all control tank cages were also removed.

Water level in all tanks remained constant during the 75-day experimental period. There were no significant differences between minimum and maximum water temperatures at the bottom of two tanks (one at each end of the array) recorded weekly. Temperatures ranged from 24 ± 4 to 34 ± 3°C over the course of the experiment. We visually censused all tanks for larvae exhibiting behavioral and phenotypic symptoms of infection (e.g., lethargy, inability to submerge, pustules on body surface, rapid degeneration of gills; Jancovich et al., 1997; Parris et al., 2004) on days 12, 44, and 75. On day 75 (21 August), we removed all remaining larvae from tanks with dip nets to assess proportion of larvae becoming cannibalistic, as metamorphosed animals do not exhibit the cannibal morphology. We thoroughly disinfected all equipment throughout the experiment and tanks at the end of the experiment by adding bleach (6% sodium hypochlorite) to yield a 10% solution.

Response Variables and Statistical Analyses.—Proportion of individuals surviving, SVL, and the proportion of survivors becoming cannibalistic were response variables. Mean values per tank were the unit of analysis because measurements from individuals within tanks were not independent. Survival was the proportion of larvae alive on day 75. SVL at day 75 served as an index of early larval growth rate, an important component of amphibian performance (Semlitsch, 1987; Ziemba and Collins, 1999). Moreover, by day 75 sublethal ATV infections may be reflected in differential growth. Larval responses were tested for the main effect of infection status using multivariate analysis of variance for the three response variables together and then with univariate analyses of variance for each response separately. Because density dependence and individual size variation may affect survival, growth rate and cannibal expression in larval salamanders (Polis, 1981), we tested for correlations among responses prior to conducting separate analyses of variance. Retrospective power analyses were performed for nonsignificant effects in analyses of variance. Frequencies were angularly transformed and SVL data log-transformed to ensure additivity of effects and homogeneity of error variances (Sokal and Rohlf, 1995). We included all responses in analyses of variance after normality was confirmed.

RESULTS

There were no significant correlations among the three larval responses (survival-SVL, $r = 0.66$, $P = 0.0742$; survival-cannibalism, $r = -0.01$, $P = 0.9826$; SVL-cannibalism, $r = 0.29$, $P = 0.4878$), thereby justifying the use of separate analyses of variance for each larval response. Multivariate larval responses were not significantly affected by infection status (Wilk's $\lambda = 0.237$, $F_{3,4} = 4.29$, $P = 0.0968$). ATV significantly reduced survival (Table 1, Fig. 1A) and reduced SVL at day 75 (Table 1, Fig. 1B). Infection status did not have a significant effect on the proportion of survivors becoming cannibalistic (Table 1, Fig. 1C). Statistical power to detect a significant infection status effect on cannibalism was high ($1 - \beta = 0.6072$). Visual censuses of tanks containing ATV-exposed larvae indicated that 18.8 ± 2.7 and 18.2 ± 2.1 (mean ± SE) larvae exhibited symptoms of infection on days 44 and 75, respectively, whereas no larvae from control tanks exhibited symptoms of infection.

DISCUSSION

Our experiment indicates that pathogens can have a strong effect on larval life-history components. Because life history traits are closely connected to fitness, ATV is likely a selective force in populations of Tiger Salamanders. ATV reduced survival by 23%, which may lower recruitment into adult salamander populations (Semlitsch

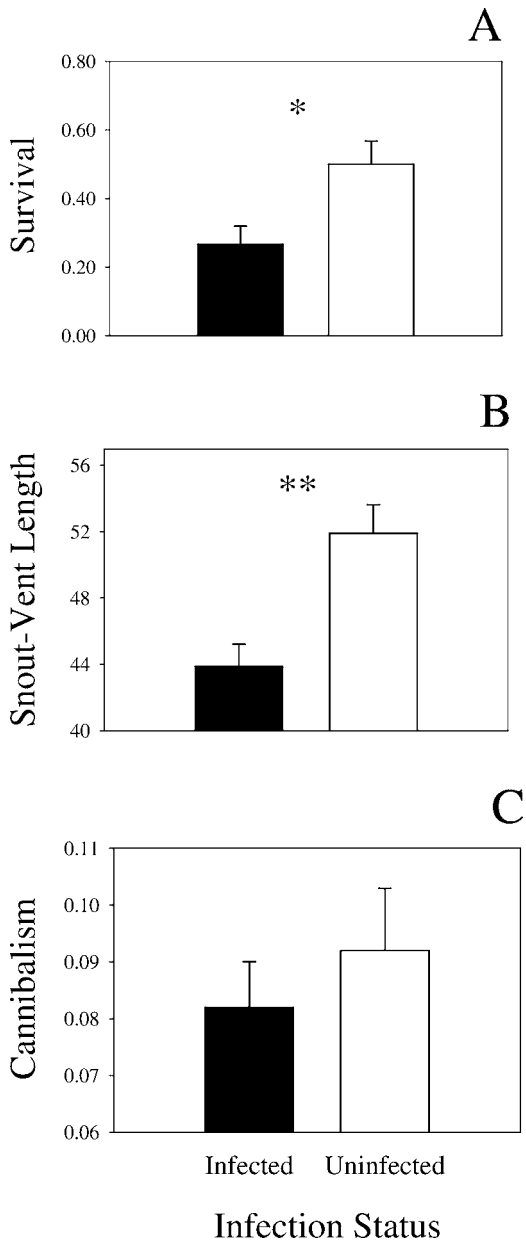

FIG. 1. (A) Proportion of individuals surviving, (B) snout–vent length (SVL) in mm at day 75, and (C) proportion of survivors becoming cannibalistic for *Ambystoma tigrinum nebulosum* larvae from the White Mountains in Arizona reared in the presence (infected) or absence (uninfected) of *Ambystoma tigrinum* virus (ATV) in experimental tanks. Values are treatment means ± 1 SE. Significant differences between infection status treatments are indicated with asterisks (*$P < 0.05$; **$P < 0.01$).

et al., 1988). The overall potential impact of the virus on salamander populations also likely includes effects on life-history traits related to growth. In virus-exposed environments, larvae exhibited reduced body size at day 75, reflecting slower growth rates. Rapid larval growth enables pond-breeding amphibians to reach minimal size necessary to escape gape-limited predators (Wilbur, 1987; Kurzava, 1998) and initiate metamorphosis (Wilbur and Collins, 1973; Alford and Harris, 1988). When larval developmental period is prolonged, exposure to aquatic predators, competitors, and water-borne diseases is increased (Semlitsch, 1987; Semlitsch et al., 1988). In addition, failure to reach a threshold size to enable metamorphosis is a cost in ephemeral habitats (Wilbur and Collins, 1973) commonly used by Tiger Salamanders. Small size at metamorphosis also can decrease survival and reproductive success in the terrestrial environment (Semlitsch et al., 1988), potentially decreasing population growth rate (Cole, 1954).

Because cannibalism increases the risk of acquiring pathogens through feeding on infected conspecifics (Pfennig, 2000), we predicted that natural selection should minimize cannibalism in environments where pathogens are present. However, ATV did not affect the frequency of cannibals produced. The lack of plasticity for cannibalism in ATV environments may be caused by an inability to discriminate between diseased and healthy individuals. Although not addressed in our study, this mechanism is consistent with Pfennig et al. (1999), who found that cannibalistic *A. t. nebulosum* larvae did not discriminate against conspecifics infected with pathogenic bacteria in choice trials. High statistical power for the nonsignificant infection status effect on cannibalism in our experiment suggests that increased experimental replication likely would not reveal a significant effect.

Lack of plasticity in cannibalism may, thus, be a result of past selection. A high frequency of infected salamanders in a population selects against cannibalism (Polis, 1981; Pfennig et al., 1998; Pfennig, 2000) and may canalize the feeding morphology of Tiger Salamander larvae in pathogen environments. Because phenotypic plasticity often carries a cost (DeWitt et al., 1998), the lack of plasticity in cannibal expression in our experiment may be caused by historically strong disease-mediated selection against cannibalism. This is supported by field data that show an inverse relationship between disease and cannibal frequency among Tiger Salamander populations throughout Arizona (Pfennig et al., 1991, 1994; Collins et al., 2003).

Although comparable to field frequencies, cannibal expression in our experiment was low given larval densities (Maret and Collins, 1994;

Ziemba et al., 2000). Although we established initial larval densities high enough to induce high cannibal production, early mortality could lower density, potentially limiting density-dependent effects, such as cannibalism. However, the lack of a significant correlation between survival and cannibalism suggests that differential survival did not affect cannibalism. Furthermore, an analysis of covariance indicated that survival explained only a small amount (4%) of the variation in proportion of survivors becoming cannibals and is, therefore, unlikely to have had an important effect on cannibal production.

It is important to note that our experiment unambiguously isolated and tested the effect of the iridovirus ATV on larval Tiger Salamander survival and life history, whereas Pfennig et al. (1991, 1998) used field-collected water or water inoculated with pathogenic bacteria in laboratory challenge trials; the presence of virus was unknown in their experiments. Because iridoviruses have been directly implicated in salamander population die-offs (Jancovich et al., 1997; Bollinger et al., 1999), it is critical to test the effects of ATV on salamander fitness independent of other naturally occurring pathogens.

Manipulative studies in seminatural environments such as experimental tanks are of great importance for understanding the ecological complexity of host-pathogen interactions. Nevertheless, judicious use of pathogens must be evaluated in outdoor environments. By maintaining low water levels and securely fastening tank lids, we minimized probability of pathogen escape. We also thoroughly disinfected all equipment and water during and after the experiment. Our cautious approach, therefore, followed proper ethical guidelines in preventing negative ecological effects (Tiedje et al., 1989; Parris and Cornelius, 2004).

Understanding how pathogens affect life-history variation is an important aspect of developing a comprehensive theory of host-pathogen biology. Furthermore, in light of mounting evidence of amphibian population declines caused by diseases (Alford and Richards, 1999; Carey, 2000; Daszak et al., 1999, 2003), it is timely to address some of the mechanisms by which disease can affect amphibians. Our results clearly demonstrate that a viral pathogen can negatively affect life-history performance in salamanders. Future studies should incorporate additional multifactorial replicated approaches to identify the potential mechanisms by which pathogens affect phenotypic and evolutionary change in their hosts.

Acknowledgments.—We thank J. Brunner and J. Roth for logistical assistance with laboratory matings and viral infections. T. Hobson, J. Beaudoin, and J. Grubaugh provided valuable help with the experimental tanks. This manuscript was improved by the comments of J. Brunner and D. Schock. We also thank the Arizona Game and Fish Department for providing collecting permits. This work was supported by National Science Foundation grants DEB 9816645 and Integrated Research Challenges in Environmental Biology grant IBN 9977063.

Literature Cited

Alford, R. A., and R. N. Harris. 1988. Effects of larval growth history on anuran metamorphosis. American Naturalist 131:91–106.

Alford, R. A., and S. J. Richards. 1999. Global amphibian declines: a problem in applied ecology. Annual Review of Ecology and Systematics 30: 133–165.

Anthony, D. W., and M. Comps. 1991. Iridoviridae. *In* J. R. Adams and J. R. Bonami (eds.), Atlas of Invertebrate Viruses, pp. 55–86. CRC Press, Boca Raton, FL.

Bollinger, T. K., J. Mao, D. Schock, R. M. Brigham, and V. G. Chinchar. 1999. Pathology, isolation, and preliminary molecular characterization of a novel iridovirus from Tiger Salamanders in Saskatchewan. Journal of Wildlife Diseases 35:413–429.

Brunkow, P. B., and J. P. Collins. 1996. Effects of individual variation in size on growth and development of larval salamanders. Ecology 77: 1483–1492.

Brunner, J. L., D. M. Schock, E. W. Davidson, and J. P. Collins. 2004. Intraspecific reservoirs: complex life history and the persistence of a lethal ranavirus. Ecology 85:560–566.

Carey, C. 2000. Infectious disease and worldwide declines of amphibian populations, with comments on emerging diseases in coral reef organisms and in humans. Environmental Health Perspectives 108:143–150.

Cole, L. C. 1954. The population consequences of life history phenomena. Quarterly Review of Biology 29:103–137.

Collins, J. P. 1981. Distribution, habitats and life history variation in the Tiger Salamander, *Ambystoma tigrinum*, in east-central and southeast Arizona. Copeia 1981:666–675.

Collins, J. P., and J. E. Cheek. 1983. Effect of food density on development of typical and cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum*. American Zoologist 23:77–84.

Collins, J. P., K. E. Zerba, and M. J. Sredl. 1993. Shaping intraspecific variation: development, ecology and the evolution of morphology and life history variation in tiger salamanders. Genetica 89:167–183.

Collins, J. P., J. L. Brunner, V. Miera, M. J. Parris, D. M. Schock, and A. Storfer. 2003. Ecology and evolution of infectious disease. *In* R. D. Semlitsch (ed.), Amphibian Conservation, pp. 137–151. Smithsonian Institution Press, Washington, DC.

Collins, J. P., J. L. Brunner, J. Jancovich, and D. M. Schock. 2004. A model host-pathogen system for studying amphibian declines: tiger salamanders

(*Ambystoma tigrinum*) and *Ambystoma tigrinum* virus. Herpetological Journal 14:195–200.

DASZAK, P., L. BERGER, A. A. CUNNINGHAM, A. D. HYATT, D. E. GREEN, AND R. SPEARE. 1999. Emerging infectious diseases and amphibian population declines. Emerging Infectious Diseases 5:735–748.

DASZAK, P., A. A. CUNNINGHAM, AND A. D. HYATT. 2003. Infectious disease and amphibian population declines. Diversity and Distributions 9:141–150.

DAY, T. 2003. Virulence evolution and the timing of disease life-history events. Trends in Ecology and Evolution 18:113–118.

DEWITT, T. J., A. SIH, AND D. S. WILSON. 1998. Costs and limits of phenotypic plasticity. Trends in Ecology and Evolution 13:77–81.

DOBSON, A. P., AND M. CRAWLEY. 1994. Pathogens and the structure of plant communities. Trends in Ecology and Evolution 9:393–398.

GEHLBACH, F. R. 1969. Determination of the relationship of tiger salamander larval populations to different stages of pond succession at the Grand Canyon, Arizona. Yearbook of the American Philosophical Society 1969:299–302.

JANCOVICH, J. K., E. W. DAVIDSON, J. F. MORADO, B. L. JACOBS, AND J. P. COLLINS. 1997. Isolation of a lethal virus from the endangered Tiger Salamander *Ambystoma tigrinum stebbinsi*. Diseases of Aquatic Organisms 31:161–167.

JANCOVICH, J. K., E. W. DAVIDSON, A. SEILER, B. L. JACOBS, AND J. P. COLLINS. 2001. Transmission of the *Ambystoma tigrinum* virus to alternate hosts. Diseases of Aquatic Organisms 46:159–163.

KIESECKER, J. M., L. K. BELDEN, K. SHEA, AND M. J. RUBBO. 2004. Amphibian decline and emerging disease. American Scientist 92:138–147.

KOELLA, J. C., P. AGNEW, AND Y. MICHALAKIS. 1998. Coevolutionary interactions between host life histories and parasite life cycles. Parasitology 116: S47–S55.

KOHLER, S. L., AND M. J. WILEY. 1997. Pathogen outbreaks reveal large-scale effects of competition in stream communities. Ecology 78:2164–2176.

KURZAVA, L. M. 1998. Tests of functional equivalence: complementary roles of salamanders and fish in community organization. Ecology 79:477–489.

LOEB, M. L. G., J. P. COLLINS, AND T. J. MARET. 1994. The role of prey in controlling expression of a trophic polymorphism in *Ambystoma tigrinum nebulosum*. Functional Ecology 8:151–158.

MARCOGLIESE, D. J., AND D. K. CONE. 1997. Food webs: a plea for parasites. Trends in Ecology and Evolution 12:320–325.

MARET, T. J., AND J. P. COLLINS. 1994. Individual responses to population size structure: the role of size variation in controlling expression of a trophic polymorphism. Oecologia 100:279–285.

———. 1997. Ecological origin of morphological diversity: a study of alternative trophic phenotypes in larval salamanders. Evolution 51:898–905.

MICHALAKIS, Y., AND M. E. HOCHBERG. 1994. Parasitic effects on host life-history traits: a review of recent studies. Parasite 1:291–294.

PARRIS, M. J. 1999. Hybridization in leopard frogs (*Rana pipiens* complex): larval fitness components in single-genotype populations and mixtures. Evolution 53:1872–1883.

PARRIS, M. J., AND T. O. CORNELIUS. 2004. Fungal pathogen causes competitive and developmental stress in larval amphibian communities. Ecology 85:3385–3395.

PARRIS, M. J., A. DAVIS, AND J. P. COLLINS. 2004. Single-host pathogen effects on mortality and behavioral responses to predators in salamanders (Urodela: Ambystomatidae). Canadian Journal of Zoology 82:1477–1483.

PEDERSEN, S. C. 1991. Dental morphology of the cannibal morph in the Tiger Salamander, *Ambystoma tigrinum*. Amphibia-Reptilia 12:1–14.

PFENNIG, D. W. 2000. Effect of predator-prey phylogenetic similarity on the fitness consequences of predation: a trade-off between nutrition and disease? American Naturalist 155:335–345.

PFENNIG, D. W., M. L. G. LOEB, AND J. P. COLLINS. 1991. Pathogens as a factor limiting the spread of cannibalism in Tiger Salamanders. Oecologia 88: 161–166.

PFENNIG, D. W., P. W. SHERMAN, AND J. P. COLLINS. 1994. Kin recognition and cannibalism in polyphenic salamanders. Behavioral Ecology 5:225–232.

PFENNIG, D. W., S. HO, AND E. A. HOFFMAN. 1998. Pathogen transmission as a selective force against cannibalism. Animal Behavior 55:1255–1261.

PFENNIG, D. W., J. P. COLLINS, AND R. E. ZIEMBA. 1999. A test of alternative hypotheses for kin recognition in cannibalistic Tiger Salamanders. Behavioral Ecology 10:436–443.

POLIS, G. A. 1981. The evolution and dynamics of intraspecific predation. Annual Review of Ecology and Systematics 12:225–251.

REILLY, S. M., G. V. LAUDER, AND J. P. COLLINS. 1992. Performance consequences of a trophic polymorphism: feeding behavior in typical and cannibal phenotypes of *Ambystoma tigrinum*. Copeia 1992: 672–679.

ROSE, F. L, AND D. ARMENTROUT. 1976. Adaptive strategies of *Ambystoma tigrinum* Green inhabiting the Llano Estacado of west Texas. Journal of Animal Ecology 45:713–729.

SEMLITSCH, R. D. 1987. Density-dependent growth and fecundity in the paedomorphic salamander *Ambystoma talpoideum*. Ecology 68:1003–1008.

SEMLITSCH, R. D., D. E. SCOTT, AND J. H. K. PECHMANN. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69: 184–192.

SHAFFER, H. B., AND M. L. MCKNIGHT. 1996. The polytypic species revisited: genetic differentiation and molecular phylogenetics of the Tiger Salamander *Ambystoma tigrinum* (Amphibia: Caudata) complex. Evolution 50:417–433.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry. 3rd ed. W. H. Freeman and Company, New York.

STORFER, A., S. G. MECH, M. W. REUDINK, R. E. ZIEMBA, J. WARREN, AND J. P. COLLINS. 2004. Evidence for introgression in the endangered Sonora Tiger Salamander *Ambystoma tigrinum stebbinsi* (Lowe). Copeia 2004:783–796.

TIEDJE, J. M., R. K. COLWELL, Y. L. GROSSMAN, R. E. HODSON, R. E. LENSKI, R. N. MACK, AND P. J. REGAL. 1989. The planned introduction of genetically engineered organisms: ecological considerations and recommendations. Ecology 70:298–315.

WASHBURN, J. O., D. R. MERCER, AND J. R. ANDERSON. 1991. Regulatory role of parasites: impact on host population shifts with resource availability. Science 253:185–188.

WILBUR, H. M. 1987. Regulation of structure in complex systems: experimental temporary pond communities. Ecology 68:1437–1452.

WILBUR, H. M., AND J. P. COLLINS. 1973. Ecological aspects of amphibian metamorphosis. Science 182:1305–1314.

ZIEMBA, R. E., AND J. P. COLLINS. 1999. Development of size structure in Tiger Salamanders: the role of intraspecific interference. Oecologia 120: 524–529.

ZIEMBA, R. E., M. T. MYERS, AND J. P. COLLINS. 2000. Foraging under the risk of cannibalism leads to divergence in body size among Tiger Salamander larvae. Oecologia 124:225–231.

Accepted: 6 April 2005.

Journal of Herpetology, Vol. 39, No. 3, pp. 372–379, 2005

Rediscovery, Redescription, and Advertisement Call of *Eleutherodactylus heterodactylus* (Miranda Ribeiro, 1937) (Anura: Leptodactylidae), and Notes on Other *Eleutherodactylus*

JOSÉ M. PADIAL AND IGNACIO DE LA RIVA[1]

Museo Nacional de Ciencias Naturales, CSIC. C/José Gutierrez Abascal 2, 28006 Madrid, Spain

ABSTRACT.—***Eleutherodactylus heterodactylus* was rediscovered in Cerrado montane forest of eastern Bolivia, 250–300 km airline from its type locality in Brazil, in similar habitat. The advertisement call is described for the first time. This species shares morphological features with species of the *Eleutherodactylus binotatus* and *Eleutherodactylus discoidalis* groups but is not assigned to either group pending further study. We confirm that *Eleutherodactylus crepitans* and *Eleutherodactylus dundeei* are valid species easily distinguishable from *Eleutherodactylus fenestratus*. Bolivian populations of the Andes previously assigned to *E. dundeei* correspond to an undescribed species allied to *Eleutherodactylus peruvianus*.**

Although a great part of the nearly 700 recognized species of *Eleutherodactylus* inhabit humid mountain forest of the Andes, the lowland Amazonian forest, the Mata Atlantica and the Caribbean islands, some of them also occur in the South American open formation of the Cerrado (Frost, 1985; D. R. Frost, Amphibian species of the world 3.0, http://research.amnh.org/herpetology/amphibia/index.php, 2004; AmphibiaWeb, http://elib.cs.berkeley.edu/aw/, 2004). Among these Cerrado species is *Eleutherodactylus heterodactylus* (Miranda-Ribeiro), a form hitherto known only from the original description, based on two specimens collected in a sandstone cave near the town of Cáceres (Matto Grosso, Brazil) (A. Miranda-Ribeiro, 1937; Heyer and Muñoz, 1999). A. Miranda-Ribeiro (1937) created the genus *Teletrema* to accommodate the species, which he called *Teletrema heterodactylum*. P. Miranda-Ribeiro (1955) designated as lectotype MN106A. Myers (1962) put *Teletrema* under the synonymy of *Eleutherodactylus* and provided some morphological notes on the two syntypes. Heyer and Muñoz (1999) studied the types and wrote: "Both types are faded such that most features of any color patterns are no longer discernible. The lectotype is in poor condition, the paralectotype is in worse condition. The paralectotype is very brittle and fragile and disintegrates more each time it is handled. The lectotype is the (noticeably) larger of the two ... ." Based on the original description and illustrations of Miranda-Ribeiro (1937), and on the examination of the specimens, Heyer and Muñoz (1999) compared *E. heterodactylus* with other *Eleutherodactylus* inhabiting Mato Grosso: *Eleutherodactylus fenestratus* (Steindachner, 1864), *Eleutherodactylus dundeei* Heyer and Muñoz 1999, and *Eleutherodactylus crepitans* Bokermann, 1965. They recognized the species status of *E. heterodactylus* and described the morphological differences with the other species. Lynch and Duellman (1997) included this species in the *Eleutherodactylus binotatus* species group, from the Atlantic Forest.

During the revision of museum specimens from Museo de Historia Natural Noel Kempff Mercado (Santa Cruz de la Sierra, Bolivia), we surprisingly found some distinctive, unidentified specimens of *Eleutherodactylus* collected in the

[1] Corresponding Author. E-mail: iriva@mncn.csic.es

semideciduous forest of the Cerrado mountain area of the Serranía de Santiago, in Eastern Bolivia. These specimens did not belong to any of the known species of *Eleutherodactylus* in Bolivia listed by De la Riva et al. (2000). This finding encouraged us to organize an expedition to the Serranía de Santiago, and we found *Eleutherodactylus* calling at night in the forest of the hills and near a sandstone cave where our colleages D. Embert and S. Reichle had mentioned the occurrence of these frogs previously. Comparisons of the specimens obtained with species from Bolivia and from the Cerrado area of Brazil led us to conclude they were *E. heterodactylus*. This is the first record after its original description (Heyer and Muñoz 1999; D. R. Frost, Amphibian species of the world 3.0, http://research.amnh.org/herpetology/amphibia/index.php, 2004) and the first time it is reported for Bolivia, 250–300 km airline distance from the type locality.

Because of the bad condition of the type specimens (Heyer and Muñoz, 1999) and the brief original description, we redescribe *E. heterodactylus* based on our new material. We also describe its advertisement call, provide some data on its biology and ecology, and discuss on its taxonomic affinities. In addition, we include some taxonomic notes on other species from the Cerrado, namely *E. crepitans* (Bokermann, 1965) and *E. dundeei* Heyer and Muñoz, 1999.

Materials and Methods

Specimens were fixed in 10% formalin and preserved in 70% ethanol. For morphological and color characteristics, we follow Lynch and Duellman (1997). Measurements were taken with a digital caliper to the nearest 0.01 mm, but following Hayek et al. (2001), we rounded all measurements to one decimal point. Measurements of the lectotype of *E. heterodactylus* are taken from Heyer and Muñoz (1999). Abbreviations are as follows: snout-vent length, SVL; head length (from rictus to tip of snout), HL; head width (measured at level of rictus), HW; interorbital distance, IOD; eye length, EL; upper eyelid width, EW; eye to nostril distance, EN; eye to eye distance (distance between the anterior margins of eyes), EE; tympanic membrane height, TYH; tympanic membrane length, TYL; width of the terminal disk of third finger, FIII; width of the terminal disk of fourth finger, FIV; thigh length, THIGH; tibia length, TL; foot length (from proximal border of inner metatarsal tubercle to tip of fourth toe), FL; width of the terminal disk of fourth toe, TIV; and forearm length (from elbow to the proximal margin of thenar tubercle), ARM. Color characteristics were taken in life. Recording equipment included a Sony WM D6C tape recorder and a Sennheiser Me 80 directional microphone. Recordings were processed on an Apple Macintosh computer. The sounds were digitized and edited at a sampling frequency of 44.1 KHz and 16 bit resolution with a Delta 66 digitizing board and Peak 3.2 (OSX) software. Raven 1.1 (Cornell University, Ithaca, New York) software was used to obtain numerical information and to generate audiospectrograms and oscillograms. Frequency information was obtained through fast Fourier transform (FFT; width, 512 points). Digitized calls were deposited in the Fonoteca Zoológica of the Museo Nacional de Ciencias Naturales (Madrid, Spain; track number 2634). Voucher specimens and material studied correspond to the herpetological collections of: Museo de Historia Natural Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia (MNKA, formely MNK and NKA); Museo de Historia Natural de la Universidad Mayor de San Marcos, Lima, Peru (MHNSM); Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN); Museo de Zoologia da Universidade de São Paulo, Brazil (MZUSP); Museu Nacional, Rio de Janeiro, Brazil (MN); Naturhistoriska Museet, Göteborg, Sweden (NHMG); and Natural History Museum, the University of Kansas, Lawrence (KU).

Systematics

Redescription of *Eleutherodactylus heterodactylus* (Miranda Ribeiro, 1937)

Teletrema heterodactylum Miranda-Ribeiro, 1937; syntypes: MN 106 (2 specimens); type locality: ''gruta dita Facendinha,'' Matto-Grosso, Cáceres, Brazil.

Teletrema heterodactylum: Miranda-Ribeiro, 1955; MN 106A, designated lectotype.

Eleutherodactylus heterodactylus: Myers, 1962.

Eleutherodactylus (*Eleutherodactylus*) *heterodactylus*: Lynch and Duellman, 1997.

Eleutherodactylus heterodactylus: Heyer and Muñoz, 1999.

Diagnosis.—A medium-sized species of *Eleutherodactylus* (Fig. 1) characterized by (1) skin on dorsum smooth, venter smooth, posterior surfaces of limbs smooth, groin granular, discoidal fold conspicuous, almost reaching the groin, no dorsolateral folds, postrictal glands well developed; (2) tympanic membrane and annulus distinct, its length about two-thirds of eye length, supratympanic fold well developed; (3) head longer than wide, snout slightly pointed in dorsal view, round in lateral profile, canthus rostralis sharp, slightly concave; (4) cranial crests absent, upper eyelid tubercles absent; (5) vomerine odontophores medial to choanae; (6) males with vocal slits and a single faint nuptial pad on thumb; (7) first finger longer than second,

FIG. 1. *Eleutherodactylus heterodactylus* MNK A7177, female (SVL, 30.79 mm) from Serranía de Santiago, Santa Cruz, Bolivia.

subarticular tubercles enlarged and subconical, supernumerary tubercles small, rounded, terminal discs of Fingers I and II rounded, not enlarged, those of Fingers III and IV markedly enlarged, ovate (Fig. 2); (8) lateral fringes and keels on fingers absent; (9) two to four ulnar tubercles small, white; (10) no tubercles on heel, small, slightly elongate proximal tubercle on tarsus; (11) inner metatarsal tubercle small, high, ovoid, outer more rounded, subequal; (12) toes lacking lateral fringes or keels, webbing absent, fifth toe shorter than third, discs of toes slightly enlarged, rounded (Fig 2); (13) dorsal coloration light reddish-brown with dark brown to black spots and marks, outlined by cream; in preservative, dorsal regions brownish-gray, snout dark gray with darker marks, throat cream with dense and fine brown mottling, venter immaculate.

Eleutherodactylus heterodactylus can be distinguished from the other species of the open formations in Mato Grosso (*E. crepitans* and *E. dundeei*) by the following combination of characters: discs of Fingers III and IV greatly enlarged, ovate, much larger than any other finger or toe disc, approximately one-half to two-thirds the size of tympanum; vomerine odontophores situated between choanae; skin of venter smooth; first finger longer than second; and absence of well-developed toe fringes and tarsal fold. The two other species mentioned have triangular, moderately enlarged discs on Fingers III and IV, and a well-developed tarsal fold (small like a tubercle and almost indistinct in *E. heterodactylus*). Additionally, *E. crepitans* and *E. fenestratus* are larger than *E. heterodactylus*; *E. fenestratus* and *E. heterodactylus* demonstrate marked sexual size dimorphism, whereas *E. crepitans* does not. *Eleutherodactylus heterodactylus* resembles in general appearance the Andean *Eleutherodactylus discoidalis* and both share an uncommon character: the vomerine odontophores are situated between the choanae; however, *E. heterodactylus* has ovate and much more enlarged discs on Fingers III and IV (moderately enlarged and truncate in *E. discoidalis*).

Description.—Head longer than wide, slightly pointed in dorsal view and rounded in lateral profile; nostrils slightly protuberant, oriented posterolaterally; canthus rostralis distinct, slightly concave; loreal region flat; upper eyelid without tubercles; no cranial crests. Supratympanic fold distinct; tympanic membrane and tympanic annulus distinct; tympanic membrane nearly round, its length about two-thirds of eye length; one to two postrictal glands. Choanae rounded, very small, anterolateral, not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; vomerine odontophores medial to choanae, very small and almost in contact. Skin of dorsal surfaces and posterior parts of hind limbs smooth, with scarce granules and/or small flat warts only on the supratympanic fold and anterior margin of the flanks; ventral skin smooth; no dorsolateral folds; ventral discoidal fold distinct, almost reaching the groin.

Two to four small ulnar tubercles; two palmar tubercles, outer rounded, almost of the same size as inner; supernumerary tubercles small, rounded, smaller than subarticular tubercles; subarticular tubercles large, subconical; discs of Fingers I and II small, rounded, those of Fingers III and IV greatly enlarged, ovate; fingers lacking lateral fringes and keels; single white nuptial pad on thumb; relative length of fingers from shortest to longest, II < IV < I < III.

Heels lacking tubercles or folds; tarsus with a proximal small, inconspicuous tubercle-like fold; inner metatarsal tubercle ovoid, longer than wide, almost the same size as conical outer; subarticular tubercles large, conical, directed forward; supernumerary tubercles small, rounded; toes lacking lateral fringes and keels; discs of toes rounded, slightly enlarged; relative length of toes, from shortest to longest, I < II < V < III < IV.

In life, dorsum light reddish-brown, with many dark brown marks (triangular interorbital, arrowlike scapular, X-like middorsal) outlined with cream; dorsal surfaces of extremities with dark brown to black bars; flanks with dark brown spots and an irregular oblique band in the anterior part; loreal region and lips cream with dark bars; canthus rostralis dark brown to black; supratympanic fold dark brown to black; tympanic membrane brown, annulus cream; iris metallic yellow. Ventral surfaces white with fine brown mottling on throat; inner surfaces of hind limbs fleshy brown. The color in preservative is similar but more brownish-gray.

Variation.—There is little variation in morphological traits and color among specimens (Table 1). The single female is larger than the males. All

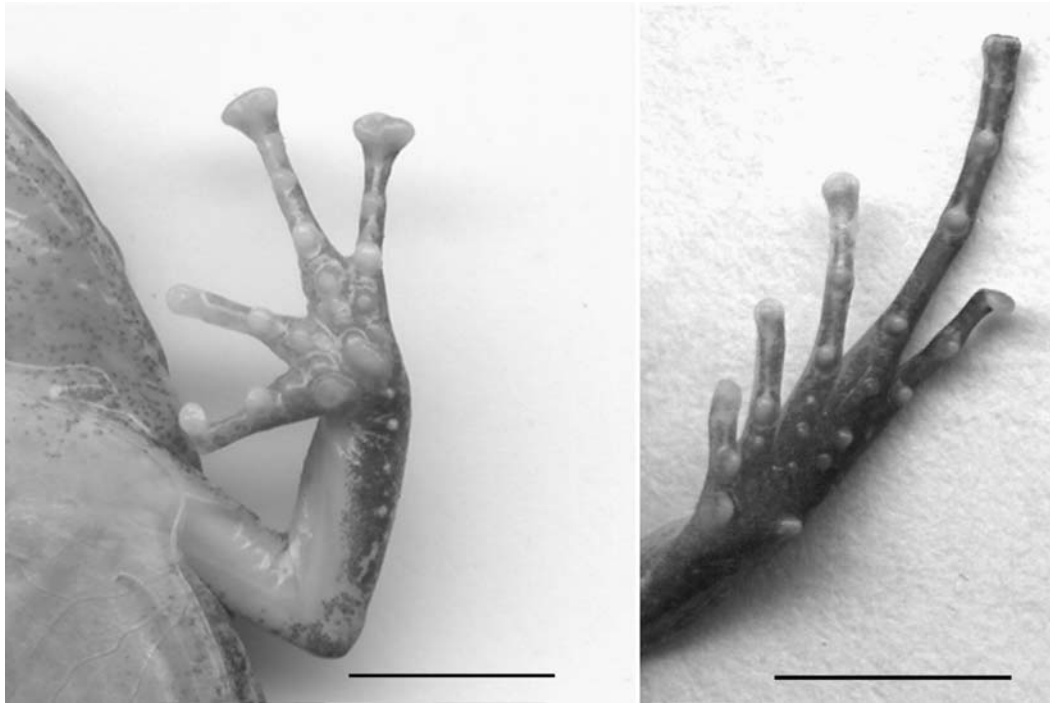

FIG. 2. Hand and foot of *Eleutherodactylus heterodactylus* MNK A7177; scale bars = 5 mm.

males have a single nuptial pad on the thumb and vocal slits. Toe fringes can be incipient (as in MNKA 6482). The contrast of the dorsal pattern varies from intense (MNKA 7175) to moderate (MNKA 7176), with more cream tonalities. A pair of occipital black spots or a "W" can be present.

Distribution and Ecology.—This species inhabits the semideciduous Cerrado forest of the mountainous regions of western Brazil and eastern Bolivia. It is only known from tree localities (Fig. 3): Fazendinha, near Pirizal, Cáceres, Mato Grosso, Brazil (type locality); Bella Boca, Province Angel Sandoval, Department Santa Cruz, Bolivia; and Cerro del Arco, Province Chiquitos, Department Santa Cruz, Bolivia. Bella Boca and Cerro del Arco are, respectively, about 250 km and 300 km airline from the type locality. Males were heard calling at night in the forest, from low

TABLE 1. Measurements (in millimeters) of the lectotype and new material of *Eleutherodactylus heterodactylus*.

	MN 5089	MNKA 6482	MNKA 6357	MNKA 6356	MNKA 7175	MNKA 7176	MNKA 7177	MNCN 495	MNCN 496
SVL	24.6	28.3	26.0	25.3	26.3	26.9	30.8	26.6	26.2
HL	8.6	10.6	11.4	11.0	10.4	10.2	11.1	10.1	9.9
HW	8.5	9.6	9.3	7.9	9.9	9.1	10.7	10.2	9.6
IOD	—	3.1	2.5	2.5	2.3	2.4	2.7	2.2	2.1
EL	—	3.3	3.4	3.5	3.9	3.5	4.2	3.5	3.3
EW	—	2.3	2.4	2.5	2.6	2.3	3.0	2.6	2.4
EN	3.5	3.1	3.2	3.5	3.1	3.0	3.7	3.3	3.4
EE	5.1	—	4.9	5.3	4.7	4.6	5.0	4.4	4.8
TYH	—	2.4	2.2	2.2	2.2	2.3	2.6	2.2	2.4
TYL	2.4	2.4	2.2	2.2	2.0	2.3	2.3	2.2	2.1
FIII	1.4	1.1	1.3	1.2	1.4	1.2	1.7	1.4	1.3
FIV	—	—	1.3	1.3	1.3	1.1	1.6	1.3	1.4
THIGH	14.1	—	9.2	10.3	13.1	11.2	13.3	12.8	12.8
TL	—	14.4	13.5	13.1	13.1	13.3	13.6	13.1	13.1
FL	12.0	12.1	12.2	11.9	11.3	12.2	12.5	12.2	11.7
TIV	0.7	—	1.0	0.8	1.1	1.0	1.0	1.0	0.9
ARM	—	—	6.1	6.0	5.8	5.9	5.9	5.5	5.6

FIG. 3. Type localities of *Eleutherodactylus crepitans* (triangle) and *Eleutherodactylus heterodactylus* (square) in Brazil, and new localities of *E. heterodactylus* in Bolivian Department of Santa Cruz (dots).

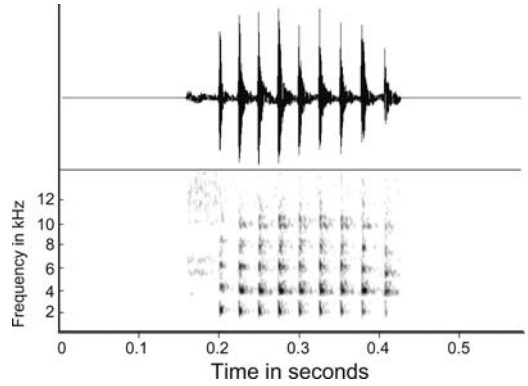

FIG. 4. Oscillogram and spectrogram of the call of *Eleutherodactylus heterodactylus* recorded at Serranía de Santiago, Santa Cruz, Bolivia; air temperature, 23°C.

vegetation or from rocks or trunks, during a dry night of the rainy season. Apparently, in this area, during the dry season, specimens can be found in sandstone caves (D. Embert and S. Reichle, pers. comm.).

The female MNKA 7177 contained seven light-orange eggs in the left oviduct and five in the right; the mean size of the eggs was 2.9 mm (range: 2.5–3.6).

Advertisement Call.—The call of *E. heterodactylus* was recorded at Serranía de Santiago, Cerro del Arco, on 4 December 2003, 2330 h; the air temperature was 23°C. Forty-three calls from four specimens were analyzed. The call is a single pulsed note averaging 176 msec (range: 126–245), 6.8 pulses (5–9), and 38.8 pulses/sec (36.5–48.6). The call has 3–5 harmonics, but the fourth and fifth harmonics are not evident in all analyses; the call repetition rate is 26.9 calls/min (24.9–29.33); the dominant frequency is 3946.9 Hz (2876–4134) and usually corresponds to the second harmonic, but exceptionally is the first harmonic, 2043.2 Hz (1981—2153; Fig. 4).

DISCUSSION

The Bolivian populations of *E. heterodactylus* are separated from the type locality by the wetlands of the Pantanal. Nevertheless, all these populations might be connected via the mountainous arc formed to the north by the low Serranías Chiquitanas, all belonging to the Precambrian shield.

Despite the simplicity of the original description of *E. heterodactylus* (Miranda Ribeiro, 1937), the drawings that accompanied it are good enough as to clearly identify the species. The characteristics of the specimens collected by us in Bolivia match well both the description and the illustrations and also are consistent with the data provided by Myers (1962) and Heyer and Muñoz (1999). The great development of the discs of Fingers III and IV, large hands, dorsal pattern, and general morphology, make it easy to distinguish *E. heterodactylus* from *E. fenestratus* or *E. dundeei*, both belonging to the *Eleutherodactylus conspicillatus* species group. Although the original description of *E. crepitans* describes the specimens from Bolivia reasonably well, examination of the type specimens of *E. crepitans* indicated that the Bolivian material did not belong to this species and that the coincidence was caused by the relative simplicity of the description. There is a possibility that the Bolivian specimens belong to an undescribed species similar to *E. heterodactylus*. The bad preservation of the types (Heyer and Muñoz, 1999), and the absence of new specimens collected at the type locality render this possibility difficult to explore. Nevertheless, considering that the Cerrado is an area of low diversity of *Eleutherodactylus* and that *E. heterodactylus* is a quite distinctive species, we consider Bolivian and Brazilian populations as conspecific; this assumption should be corroborated by collecting of new specimens at the type locality and comparisons of them with Bolivian material.

The assignment of *E. heterodactylus* to any of the species groups of *Eleutherodactylus* currently recognized is not possible with the information at hand. Lynch and Myers (1983) placed this species within the *Eleutherodactylus fitzingeri* group, and Lynch and Duellman (1997) included it in the

Eleutherodactylus binotatus species group, from the Atlantic Forest, something that Heyer and Muñoz (1999) considered lacking zoogeographic sense. We found that this species shares morphological features with species of the *E. binotatus* (Duellman and Lynch, 1997) and *E. discoidalis* (sensu Lynch, 1989) groups. However, these groups are defined mainly on the basis of plesiomorphic characters, and we prefer not to include *E. heterodactylus* in any group until the intrageneric phylogenetic relationships are better understood.

Besides *E. heterodactylus*, *E. crepitans* and *E. dundeei* also inhabit the Cerrado mountain areas of Mato Grosso. *Eleutherodactylus crepitans* is known only from the type locality (''São Vicente, Cuiabá, Mato Grosso, Brasil''). Heyer and Muñoz (1999) removed this species from the synonymy of *E. fenestratus*, where it had been placed by Lynch (1980). Lynch and Duellman (1997) did not assign it to any species group. The holotype and paratypes were collected in a dry area covered by grass-scrub vegetation in the middle of large blocks of granite (Bokermann, 1965). *Eleutherodactylus dundeei* belongs to the *Eleutherodactylus conspicillatus* species group. It was described from gallery forest of the Cerrado region of Chapada dos Guimaraes (Heyer and Muñoz, 1999).

We examined the type specimens of *E. dundeei* and *E. crepitans* and agree with Heyer and Muñoz (1999) that these are valid species well distinguishable from *E. fenestratus*. Köhler (2000) examined paratype specimens of *E. dundeei* and compared a single call from the type locality with calls of certain Bolivian specimens of *Eleutherodactylus*. Based on this, he reported *E. dundeei* for the humid Andean Amazonian slopes of Departmento de Santa Cruz, Bolivia, approximately 850 km airline from the type locality and in a completely different kind of habitat. Köhler (2000) failed to observe that those Bolivian specimens lack basal webbing on toes, possessed by all type specimens of *E. dundeei*. Although preliminary comparisons indicated that calls were similar between both populations (Köhler, 2000), a more comprehensive analysis is required (Reichle provided recordings of these frogs from Paractito, Departamento Cochabamba [2002]). Convergences in general morphology and advertisement calls are common in anurans including many *Eleutherodactylus* species, and this could explain the superficial similarity of *E. dundeei* and Andean populations assigned to this species. Furthermore, occurrence of *E. dundeei* in humid rain forest at the foot of the Andes is biogeographically discordant. No other anuran species has a disjunct distribution in the open formations of the Cerrado and in the humid rain forests at the foot of the Andes; the anuran fauna of the intervening areas (wet savannas and semihumid transitional rain forests in Beni and Santa Cruz Departments) is fairly well known, and no species of *Eleutherodactylus* occurs there (De la Riva, 1993a; De la Riva et al., 2000). Bolivian populations of *Eleutherodactylus* treated as *E. dundeei* by Köhler (2000) had been traditionally considered to be *E. fenestratus* (see De la Riva, 1993b); later, they were identified as *E. peruvianus* by De la Riva (1994), based on the examination of large Peruvian samples identified as *E. peruvianus*, from seven localities in central and southern Peru (see Appendix 1). Fifteen of 78 Peruvian specimens (19.2%) from different localities had morphology and color patterns equal to those of Bolivian specimens. It seems that populations from northern Peru and Ecuador consistently show a well-marked dorsal pattern, presence of orange spots on the posterior surface of thighs and dorsolateral folds (Lynch and Duellman, 1980; Rodríguez and Duellman, 1994; Duellman and Pramuk, 1999), whereas populations from southern Peru and Bolivia often have a less marked pattern and lack orange spots and dorsolateral folds (De la Riva, 1994; De la Riva et al., 2000). Thus, these characters are subject to considerable variation in what is currently known as *E. peruvianus*.

Köhler (2000) and Padial et al. (2000) examined the holotype of *E. peruvianus* (NHMG 490, type locality: Roque, Departamento San Martín, Peru; indicated as NHMG type number 0030:1 by Padial et al. [2000]). Köhler (2000) concluded that the absence of dorsolateral folds in Bolivian specimens and differences in finger lengths and coloration clearly indicate that they belong to a different species, which he considered to be *E. dundeei*; consequently, he deleted *E. peruvianus* from the list of anurans of Bolivia, although he did not discard its presence in that country. However, he examined only the holotype but not a large sample of specimens to assess the range of variation of these characters, a range into which Bolivian specimens fall perfectly. We consider plausible that more than one species is involved in what is currently known as *E. peruvianus*. If this is the case, then the Bolivian populations might represent an undescribed species related to *E. peruvianus*; this, however, would not exclude the possibility that ''true'' *E. peruvianus* also occur in the country, as suggested by Köhler (2000). Until a taxonomic study is done to confirm or discard the presence of more than one species, the mentioned populations from Bolivia and central and southern Peru should be referred to as *E. peruvianus* or *E.* cf. *peruvianus*; at the same time, *E. dundeei* should be deleted from the list of amphibians of Bolivia, although its presence is still plausible in the Cerrado formations of northeastern Santa Cruz (De la Riva et al., 2000).

A species similar to *E. peruvianus* s. l. is *Eleutherodactylus danae* Duellman, from which *E. peruvianus* was purported to differ from by having the skin of the venter smooth and the first finger longer than the second (Duellman, 1978). Comparisons of the holotype of *E. danae* (KU 162307) with the previously mentioned large samples identified as *E. peruvianus* render the diagnosis of *E. danae* quite inconsistent (IDLR, pers. obs.). This fact is probably due to the existence in these samples of the putative new species mentioned above, which would be less different from *E. danae* than the true *E. peruvianus*. *Eleutherodactylus danae* is indeed a valid species with a distinctive advertisment call (Köhler and Jungfer, 1995), and it inhabits the Andean cloud forests of Peru and Bolivia; *E. danae* and *E. peruvianus* might be sympatric at intermediate elevations.

Acknowledgments.—JMP was under grant support from the Mutis program of the MAE-AECI (Spain). The travel to the U.S. museums was founded by the Ernst Mayr Travel Grant in Animal Systematics (Museum of Comparative Zoology, Harvard University) to JMP. We are grateful to the personnel at the Museo de Historia Natural Noel Kempff Mercado, especially to M. Suárez, R. Vespa, A. Justiniano, R. Montaño, L. Gonzáles, and J. Aparicio (Colección Boliviana de Fauna), by providing working facilities and loaning of specimens. We thank L. Gonzáles (MNKM), S. Reichle (TNC), and D. Embert (ZFMK) for providing specimens and/or field notes. We thank H. Zaher and P. Vanzolini (MZUSP), R. Heyer (National Museum of Natural History, Smithsonian Institution), J. Córdova and C. Aguilar (MHNSM), and J. Hanken (MCZ) for providing space at their institutions for examination of specimens. R. Márquez and G. Solís (MNCN) helped us with the sound analyses. R. Heyer provided valuable comments and loaned recordings of *E. peruvianus*. This work was partially funded by the project REN/GLO 2001-1046 of the Spanish Ministry of Science and Technology (IDLR, Principal Investigator).

Literature Cited

Bokermann, W. C. A. 1965. Três novos batráquios da regiao central de Mato Grosso, Brasil (Amphibia, Salientia). Revista Brasileira de Biologia 25:257–264.

De la Riva, I. 1993a. Ecología de una comunidad neotropical de anfibios durante la estación lluviosa. Unpubl. Ph.D. diss., Universidad Complutense de Madrid, Madrid, Spain.

———. 1993b. Sinopsis del género *Eleutherodactylus* (Amphibia, Anura, Leptodactylidae) en Bolivia y adición de tres especies nuevas para el país. Revista Española de Herpetología 7:97–105.

———. 1994. Geographic Distribution. *Eleutherodactylus peruvianus* (NCN). Herpetological Review 25:159.

De la Riva, I., J. Köhler, S. Lötters and S. Reichle. 2000. Ten years of research on Bolivian amphibians: updated checklist, distribution, taxonomic problems, literature and iconography. Revista Española de Herpetología 14:19–164.

Duellman, W. E. 1978. New species of leptodactylid frogs of the genus *Eleutherodactylus* from the Cosñipata Valley, Perú. Proceedings of the Biological Society of Washington 91:418–430.

Duellman, W. E., and J. B. Pramuk. 1999. Frogs of the genus *Eleutherodactylus* (Anura: Leptodactylidae) in the Andes of northern Peru. Scientific Papers of the Natural History Museum, Univ. of Kansas 13:1–78.

Frost, D. R. (ed.). 1985. Amphibian Species of the World. A Taxonomic and Geographical Reference. Allen Press and Association of Systematics Collections, Lawrence, KS.

Hayek, L. A., W. R. Heyer, and C. Gascon, C. 2001. Frog morphometrics: a cautionary tale. Alytes. 18:153–177.

Heyer, W. R., and A. M. Muñoz, 1999. Validation of *Eleutherodactylus crepitans* Bokermann, 1965, notes on the types and type locality of *Telatrema* [sic] *heterodactylum* Miranda-Ribeiro, 1937, and description of a new species of *Eleutherodactylus* from Mato Grosso, Brazil (Amphibia: Anura: Leptodactylidae). Proceedings of the Biological Society of Washington 112:1–18.

Köhler, J. 2000. Amphibian diversity in Bolivia: a study with special reference to montane forest regions. Bonner Zoologische Monographien 48:1–243.

Köhler, J., and K. H. Jungfer. 1995. Eine neue Art und ein Erstnachweis von Fröschen der Gattung *Eleutherodactylus* aus Bolivien. Salamandra 31:149–156.

Lynch, J. D. 1980. A taxonomic and distributional synopsis of the Amazonian frogs of the genus *Eleutherodactylus*. American Museum Novitates 2696:1–24.

———. 1989. Intrageneric relationships of mainland *Eleutherodactylus* (Leptodactylidae). I. A review of the frogs assigned to the *Eleutherodactylus discoidalis* species group. Contributions in Biology and Geology, Milwaukee Public Museum 79:1–25.

Lynch, J. D., and W. E. Duellman. 1980. The *Eleutherodactylus* of the Amazonian slopes of the Ecuadorian Andes (Anura: Leptodactylidae). Univ. of Kansas Museum of Natural History Miscellaneous Publications 69:1–86.

———. 1997. Frog of the genus *Eleutherodactylus* in western Ecuador: systematics, ecology, and biogeograpy. Univ. of Kansas Natural History Museum Special Publications 23:1–236.

Lynch, J. D., and C. W. Myers. 1983. Frogs of the fitzingeri group of *Eleutherodactylus* in Eastern Panama and chocoan South America. Bulletin of the American Museum of Natural History 175: 481–572.

Miranda-Ribeiro, A. 1937. Alguns batrachios novos das collecçôes do Museu Nacional. O Campo, May 1937:66–69.

Miranda-Ribeiro, P. 1955. Tipos das espécies e subespécies do Prof. Alipio de Miranda Ribeiro depositados no Museu Nacional (Com una relação dos gêneros, espécies e subespécies descritos). Arquivos do Museu Nacional, Río de Janeiro 42:389–417.

MYERS, G. S. 1962. The American leptodactylid frog genera *Eleutherodactylus*, *Hylodes* (= *Elosia*), and *Caudiverba* (= *Calyptocephalus*). Copeia 1962:195–202.

PADIAL, J. M., S. BIELSKIS, AND J. CASTROVIEJO. 2000. Sobre los anfibios del pie de monte del Departamento de La Paz (Bolivia). Boletín de la Asociación Herpetológica Española 11:59–62.

REICHLE, S. 2002. *Eleutherodactylus* cf. *peruvianus*. *In:* R. Márquez, I. De la Riva, J. Bosch, and E. Matheu (eds), Guía sonora de las ranas y sapos de Bolivia. ALOSA-AHE-MNCN, Barcelona, Spain.

RODRÍGUEZ, L. O., AND W. E. DUELLMAN. 1994. Guide to the frogs of the Iquitos Region, Amazonian Peru. Univ. of Kansas Museum of Natural History Miscellaneous Special Publication 22:1–80.

Accepted: 8 April 2005.

APPENDIX 1

Specimens Examined

Eleutherodactylus crepitans: BRAZIL: Mato Grosso, São Vicente, MZUSP 85628 (holotype), MZUSP 73671 (allotype, same data as holotype).

Eleutherodactylus danae: PERU: Dept. Cusco, Río Cosñipata, 4 km (by road) southwest of Santa Isabel, KU 162307 (holotype).

Eleutherodactylus dundeei: BRAZIL: Mato Grosso, Rio Coxipozinho, Veu da Noiva, Chapada dos Guimarães, MZUSP 79834–7 (type series).

Eleutherodactylus heterodactylus: BOLIVIA: Dept. Santa Cruz, Prov. Angel Sandoval, Bella Boca (17°50′S, 59°00′W), MNKA 6482. Dept. Santa Cruz, Prov. Chiquitos, Serranía de Santiago, Cerro del Arco (18°20′50.7″S, 59°53′37.06″W), MNKA 6356–7, 7175–7, MNCN 42014–5.

Eleutherodactylus cf. *peruvianus*: BOLIVIA: Dept. Santa Cruz, Prov. Andrés Ibáñez, Espejillos (17°50′S, 63°25′W), MNKA 7172–4, MNCN 42017–8. Dept. Santa Cruz, Prov. Ichilo, La Chonta (17°39′36″S, 63°42′06.6″W), MNCN 42016. PERU: Dept. Cusco, approximately 40 km east Quincemil on Puerto Maldonado road above Marcapata, KU 196475. Dept. Huánuco, Casa Campa, southern slope of Serranía de Sira, KU 154848–52. Dept. Huánuco, Laguna, 1280 m, southern slope of Serranía de Sira, KU 154863–65. Dept. Huánuco, Río Llullapichis, 4–5 km upstream from Río Pachitea, KU 154835–47, 154858–62, 171867–91. Dept. Madre de Dios, Cocha Cashu, P. N. Manu, KU 154856–57. Dept. Madre de Dios, Cuzco Amazónico, KU 194909, 205107, 205132–5, 205137–8, 205142, 207715–7, 215481–8. Dept. Madre de Dios, Manu, 365 m, KU 154853–5.

Eleutherodactylus peruvianus: PERU: Dept. San Martín, Roque, NHMG 490 (holotype).

Journal of Herpetology, Vol. 39, No. 3, pp. 379–383, 2005

New Species of *Clelia* (Colubridae) from the Inter-Andean Dry Valleys of Bolivia

STEFFEN REICHLE[1,2] AND DIRK EMBERT[2]

[1]*The Nature Conservancy–Bolivia Program, Edificio Multicentro Torre B, piso 4 ofc. 402, La Paz, Bolivia*
[2]*Zoologisches Forschungsinstitut und Museum Alexander Koenig, Adenauerallee 160 D-53113 Bonn, Germany*

ABSTRACT.—A new species of *Clelia* Fitzinger, 1826, is described on the basis of 37 specimens. It differs from all other *Clelia* by having two loreals and a higher number (21 vs. 19) of dorsal scale rows in the neck region. The species is probably endemic to the Bolivian inter-Andean dry valleys.

RESUMEN.—Una nueva especie de *Clelia* Fitzinger, 1826 es descrita sobre la base de 37 especimenes. Se diferencia de todas las otras *Clelia* en presentar dos loreales y un número más alto (21 vs. 19) de hilas de escamas en la región nucal. La especie muy probablemente es endémica para los valles secos interandinos de Bolivia.

In his revision of the genus *Clelia* Zaher (1996) divided the genus into *Clelia* and *Boiruna*, recognizing nine species among the two genera. Since then, three new species of *Clelia* (*Clelia quimi*, *Clelia Montana*, and *Clelia hussami*) have been described by Franco et al. (1997) and Morato et al. (2003). The genus *Clelia* is distributed throughout most of Latin America, from Mexico to Argentina on the mainland and occurs on Trinidad and the Lesser Antillean Islands of Grenada and St. Lucia (Zaher, 1996). To date, two species have been reported from Bolivia, *Clelia clelia* and *Clelia rustica* (Fugler, 1986; Scrocchi and Viñas, 1990). All specimens of *Clelia* in the Bolivian collections (MNKR, CBF) and one specimen deposited in Bonn, Germany (ZFMK), labeled as *C. rustica*, differ from the taxon described herein. Comparing the 37 specimens with data from all other pseudoboine species revealed they belong to a new species of *Clelia*.

Materials and Methods

Ventral count methodology follows that of Dowling (1951). For further comparisons we adopted the sequence of standard characters used by Zaher (1996) for xendodontine snakes. Abbreviations used are TTL (total length); TL (tail length); HL (head length, measured from tip of snout to furthest edge of posterior-most supralabial); HW (head width, measured at angle of jaw); and ED (eye diameter, measured horizontally at is midpoint). TTL and Tl were measured to the nearest 0.1 cm; all other measurements were made to the nearest 0.1 mm using a caliper held under a dissecting microscope. Sex was determined by observation of anatomical structure at the base of the tail through a small ventral incision. Furthermore, we examined the stomach and oviduct by opening with a scalpel. Because the local human population collects snakes randomly and stores the collected specimens in the ''Noel Kempff Mercado'' Museum of Natural History the date of collection was taken as an indicator for time of activity. Abbreviations of scientific collections used are MNKR (Museo Noel Kempff Mercado,Bolivia, Reptiles), CBF (Colección Boliviana de Fauna, Bolivia), ZFMK (Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany), ZSM (Zoologische Staatssammlung München, Germany).

Clelia langeri sp. nov.
Figure 1–2

Holotype.—Museo Historia Natural Noel Kempff Mercado, Santa Cruz, Bolivia (MNKR) 867; an adult male from the area of Villa Merced (18°6′S, 64°11′W), Province Florida, Department of Santa Cruz, Bolivia collected by Frey Andrés Langer op. on 14 April, 1994. The area lies in about 1300 m altitude in the inter-Andean dry valleys of Bolivia.

Paratypes.—Bolivia: Department Santa Cruz: Province Florida: Pampagrande: MNKR 857 female collected 14 March 1996, MNKR 985 male collected 16 March 1996, MNKR 988 juvenile collected 3 October 1996, MNKR 1499 female collected 4 January 1998, MNKR 1500 female collected 16 January 1998, MNKR 1532 female collected 5 January 1998, MNKR 1538 male collected 10 December 1997, MNKR 1585 juvenile collected 1 October 1997, MNKR 2168 male collected 5 June 2000, MNKR 2169 female collected 17 April 2000, MNKR 2176 male collected 24 January 2000, MNKR 2177 female collected 21 May 2000, MNKR 2271 juvenile collected 31 October 2000, MNKR 2362 male collected 14 November 1999, MNKR 2509 male collected 30 June 2001, MNKR 2902 male collected 7 October 2001, MNKR 3226 female collected 27 May 2002, MNKR 3234 female collected 18 April 2002, MNKR 3491 female collected 7 March 2003; Bermejo: MNKR 2622 juvenile collected 28 November 2000; Recodo: ZFMK 75023 male collected 1 July 2001; Pacay: MNKR 2170 female collected 30 September 2000; Caraparial: MNKR 1708 juvenile collected 16 April 1997; Aguaclara: MNKR 1264 male collected 2 January 1996, MNKR 1534 female collected 22 September 1997; Algodonal: MNKR 859 sex undetermined collected 23 March 1996, MNKR 1221 juvenile collected 23 November 1996, MNKR 1258 female collected 24 November 1995; Zanjón: MNKR 1095 female collected 23 January 1997, MNKR 1690 juvenile collected 10 August 1997, MNKR 3235 male collected 18 April 2002; Mairana: MNKR 1225 juvenile collected 18 June 1996; Mataral: MNKR 3499 sex undetermined collected 10 February 2003; Villa Merced: MNKR 1574 sex undetermined collected 5 November 1997, MNKR 2178 male collected 9 May 2000; Department Chuquisaca: Province: Hernando Siles, Rio Pili-Pili: CBF 975 female collected 2 May 1998.

Diagnosis.—*Clelia langeri* can be distinguished from all other members of the genus by having two loreals instead of one (Fig. 1). Furthermore 36 of 37 specimens present 21 scale rows in the neck region, whereas all other *Clelia* species have only 19. Together with *C. rustica* this is the only species of the genus where juveniles do not undergo ontogenetic color pattern change.

Comparisons.—*Clelia langeri* can be distinguished from *Clelia scytalina*, *Clelia equatoriana*, and *Clelia errabunda* by the presence of 19 rows of middorsal scales, whereas these other species have 17.

Clelia langeri is distinguishable from *Clelia clelia* (Balley, 1970) by having mostly eight (rarely nine) supralabials, whereas *C. clelia* has seven, rarely eight (Balley, 1970; Cei, 1986; Cunha and Nascimiento, 1978; Scrocchi and Viñas, 1990). Neonates of *C. langeri* are uniformly brown colored, whereas those of *C. clelia* show a distinct red coloration, a white collar and a black head. Adults of *C. langeri* are dorsally uniformly brown colored, whereas those of *C. clelia* are dorsally uniformly black.

Clelia langeri can be distinguished from *Clelia bicolor* by the presence of a much higher number of ventral scales: in males 226–250 versus 176–175 (Franco et al., 1997), in females 235–255 versus 174–185 (Franco et al., 1997). Furthermore, *C. langeri* presents more subcaudal scales than *C. bicolor*, in males 87–105 versus 64–68 (Franco et al., 1997), in females 82–97 versus 57–61 (Franco et al., 1997).

Clelia langeri is distinguishable from *C. rustica* by the presence of a much higher number of subcaudal scales: in males 87–105 versus 54–64 (Zaher, 1996; Franco et al., 1997), in females 82–97 versus 49–56 (Zaher, 1996) and 44–54 (Franco

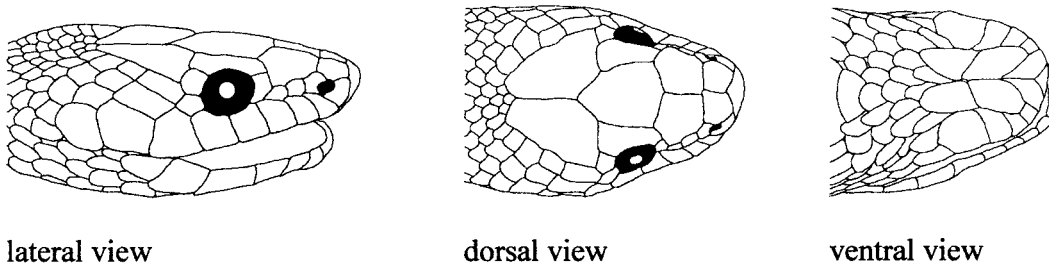

FIG. 1. Head scalation of *Clelia langeri* sp. nov. (Holotype MNKR 867) from Bolivia, Department Santa Cruz, Province Florida, Villa Merced.

et al., 1997). *Clelia langeri* also presents a higher number of ventral scales: in males 226–250 versus 190–208 (Zaher, 1996) or 201–212 (Franco et al., 1997), in females 235–255 versus 213–231 (Zaher, 1996) or 214–225 (Franco et al., 1997).

Clelia langeri can be distinguished from *Clelia hussami* by the presence of a much higher number of subcaudal scales: in males 87–105 versus 49–56 (Morato et al., 2003), in females 82–97 versus 47–50 (Morato et al., 2003). *Clelia langeri* also presents a higher number of ventral scales: in males 226–250 versus 212–225 (Morato et al., 2003), in females 235–255 versus 204–210 (Morato et al., 2003). Additionally, *C. langeri* never exhibits the narrow dark vertebral line as found in adults of *C. hussami* and neonates lack a clear nucal collar as described for *C. hussami*.

Clelia langeri can be distinguished from *Clelia quimi* and *Clelia montana* by the higher number of ventral scales present: in males 226–250 versus 184–200 in *C. quimi* and versus 201–211 in *C. montana* (Franco et al., 1997), in females 235–255 versus 193–207 in *C. quimi* and versus 209–218 in *C. montana* (Franco et al., 1997). *Clelia langeri* also can be distinguished from *C. quimi* and *C. montana* by the higher number of subcaudal scales: in

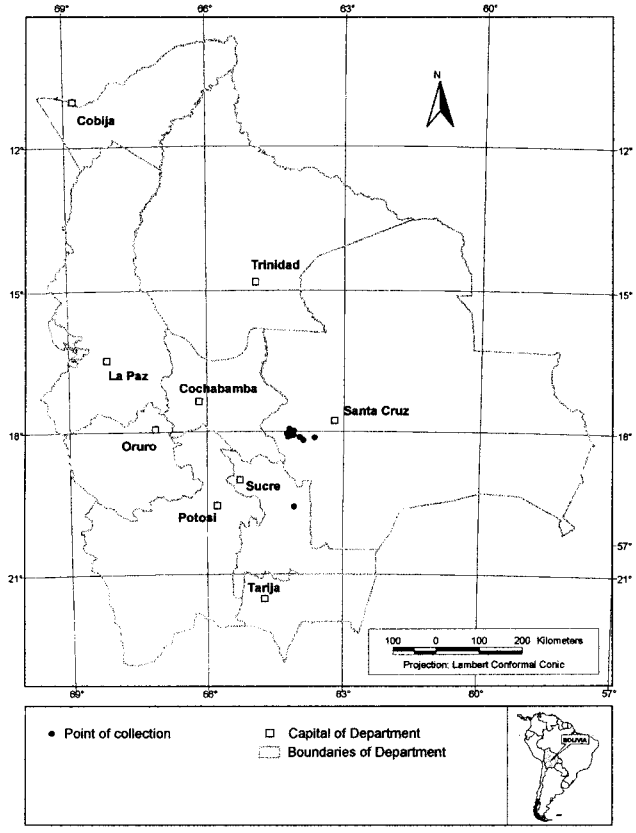

FIG. 2. Distribution of *Clelia langeri* sp. nov.

males 87–105 versus 66–79 in *C. quimi* and versus 53–57 in *C. montana* (Franco et al., 1997), in females 82–97 versus 58–71 in *C. quimi* and versus 46–49 in *C. montana* (Franco et al., 1997).

Clelia langeri can be distinguished from *Clelia plumbea* by the presence of spines on the hemipenis and the presence of two loreals these never being totally absent (loreals totally absent in 27% of the specimens of *C. plumbea*; Zaher, 1996).

Description of Holotype.—An adult male (MNKR 867), preserved in 70% alcohol. Head little distinct from neck, length 30 mm; greatest HW 20 mm; HW:HL ratio = 0.67; TTL = 940 mm; TL = 222 mm; TL:TTL ratio = 0.24. Dorsal scales smooth with two apical pits; dorsal scales in 21/19/17 rows; ventrals 238; preventrals 3. Cloacal scale undivided; subcaudals in 100 pairs. Head scutellation typical of other colubrids; rostral shield wider than high (ratio = 0.68); internasals slightly rounded, more than one-third shorter than prefrontals; prefrontals pentagonal, slightly broader than long, each in contact with internasal, nasal, both loreals, preocular, supraocular and frontal; frontal hexagonal, anterior side somewhat rounded, longer than wide, and shorter than parietals; supraoculars approximately three times as long as wide, narrower anteriorly, not protruding over eyes; parietal length/width ratio = 1.45; interparietal suture as long as frontal scale, as long as distance from frontal to tip of snout; nasal divided, longer than high; loreals rectangular as long as high; one preocular, twice as high as long; two postoculars on both sides, lower one two-thirds the size of upper one; temporals 2 + 3 + 3 on right side, 2 + 3 + 2 on the left side, the first two always two times longer than posterior ones; supralabials 8/8, second in contact with first loreal (left side), both loreals (right side), third in contact with second loreal and preocular (both sides), fourth and fifth in contact with the eye, the posteriormost four larger than the anterior ones; infralabials 8/8, first pair in contact on distal border of mental, first four in contact with anterior chin shields, and fifth (and largest pair) contacting posterior chin shields; posterior chin shields shorter than anterior ones. ED 3.3 mm, distance from eye to tip of snout 7.0 mm, ratio = 0.47; pupil vertical; dentition: maxillare 12, mandibulare 9. Dorsum light brown becoming progressively lighter on the lateral part of the body and the tip of the scales. Venter cream-colored; paraventral coloring slightly invading the lateral parts of the ventrals. Dorsum of head the same color as the body; posterior five supralabials cream colored, the three posterior with most dorsum brown coloration invading the upper edge, the anterior three brownish colored. Infralabials and the mental region the same light color as the venter.

Variation.—One specimen differs in having a dorsal scale formula of 19-19-17 instead of 21-19-17. In all other diagnostic scales it is similar to the holotype of the new species. The number of ventral scales ranges from 226–255 with an average of 241. Males 226–250 (mean = 237) and females 235–255 (mean = 244). Three preventrals, exceptions with one or two. The number of subcaudal scales ranges from 80 (one juvenile) to 105 (mean = 93). Males with more (87–105, mean = 97) than females (82–97, mean = 90). All specimens have eight supralabial scales, at least on one side (three specimens have nine on one side), eight or nine infralabial scales, two specimens present on one side only one loreal scale. The examined specimens have 2 + 3 + 3 temporals with some minor variations, for example: 2 + 2 + 3, 2 + 3 + 2 or 2 + 3 + 4. The largest male has a total length of 1128 mm, the largest female of 1295 mm, the smallest juvenile is 425 mm. The dentition of the maxilla varies between 12 and 13 teeth. Coloration in alcohol does not show significant differences to live color. The colors are just a bit brighter. There is no ontogenetic variation of coloring pattern known; juveniles show slightly brighter colors than adults.

Hemipenial Morphology.—The species presents a bilobed hemipenis with two pairs of large extrasulcular spines on each side, two intrasulcular spines, and a deeply forked sulcus spermaticus dividing on the proximal region of the hemipenial body. Each branch of the centrifugal sulcus spermaticus extends from the distal region of the body to the tip of each lobe.

Etymology.—The specific epithet langeri is in honor of Fray Andrés Langer op., a Dominican monk who started collecting reptiles in the area nine years ago and together with his collaborators from the local population has contributed more than 1400 specimens to the herpetological scientific collection in the Museum of Natural History Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia.

Distribution.—*Clelia langeri* is only known from the inter-Andean dry valleys of Bolivia in the Departments of Santa Cruz and Chuquisaca (Fig. 2). It is suspected that the distribution includes the dry valleys in the Department of Tarija.

Natural History.—Little is known about the natural history of this species. The habitat is anthropogenically modified. The altitudinal distribution is between 900 and 1500 m.a.s.l. The species shows a year-round activity pattern; juveniles were collected in, May (1) June (1), August (1), October (3), and November (3). A mouse (Muridae) was found in one of the specimens.

Remarks

The hemipenial structure shows affiliation to the colubrid subtribe Pseudoboini. Following

Zaher (1999) the hemipenial structure of the new species indicates it belongs to the genus *Clelia*. Franco et al. (1997) discussed the definition of the genus *Clelia* and *Oxyrhopus* and indicated that ontogenetic color and pattern changes are always present in *Clelia* and always absent in *Oxyrhopus*. *Clelia langeri* does present a slight ontogenetic color change but no ontogenetic pattern changes. Furthermore, populations of *Oxyrhopus formosus* in Bolivia do present a strong ontogenetic pattern change. A possible difference between the two genera, concerning their coloration, is the presence of banded or semibanded patterns at least in one of the life stages in *Oxyrhopus* but absent in *Clelia*.

Ibisch and Böhme (1993) as well Ibisch and Gross (1993) discussed possible reasons for a relative high number of endemics for the inter-Andean dry valleys of Bolivia. In addition to *Amphisbaena cegei*, *Apostolepis multicincta*, *Micrurus serranus* and *Mabuya cochabambae*, *C. langeri* is the fifth endemic reptile species found in this area.

Acknowledgments.—We thank the Dirección General de Biodiversidad (DGB) for issuing collection permits and the staff of the Noel Kempff Mercado Museum Historia Natural (MHNNKM) for the opportunity to work in the exceptional reptile collection and for use of facilities. A special thank goes to L. Gonzáles and R. Montaño for their constant assistance. Our biggest thanks must go to the Dominican Monk Fray Andres Langer op and the population of the Provincia Florida, who for the past nine years, have collected animals and plants for the MHNNKM and whose collection of reptiles forms more than a third of the whole reptile collection. For designing the map, we thank our friend C. Nowicki. We thank J. Aparicio for the opportunity to revise the collection in the CBF La Paz, Bolivia, and W. Böhme for the access to the collection in Bonn, Germany. Finally, we thank N. Acheson for his useful comments on the manuscript.

Literature Cited

Bailey, J. R. 1970. *Clelia*. *In* J. A. Peters and B. Orejas Miranda (eds.), Catalogue of the Neotropical Squamata: Part I. Snakes, pp. 62–64. Bulletin U.S. National Museum 297, Washington, DC.

Cei, J. M. 1986. Reptiles del centro, centro-oeste y sur de la Argentina. Herpetofauna de las zonas áridas y semiáridas. Museo Regionale di Scienze Naturali Monografie IV, Florence, Italy.

da Cunha, O. R., and F. P. do Nascimiento. 1978. Ofídios da Amazonia, X- As cobras da região leste do Pará. Belém, Publicações Avulsas do Museu Paraense Emílio Goeldi 31, Belem, Brazil.

Dowling, H. G. 1951. A proposed system for counting ventrals in snakes. British Journal of Herpetology 1:97–99

Franco, F. L., O. A. V. Marques, and G. Porto. 1997. Two new Species of Colubrid Snakes of the Genus *Clelia* from Brazil. Journal of Herpetology 31: 483–490.

Fugler, C. M. 1986. Una Lista preliminar de las Serpientes de Bolivia. Ecologia en Bolivia 8:45–72.

Ibisch, P., and W. Böhme. 1993. Zur Kenntnis der innerandinen Herpetofauna Boliviens (Provinz Arque, Departamento Cochabamba). Herpetofauna 15:15–26.

Ibisch, P., and E. Gross. 1993. *Puya prosanae*, a New Dwarf *Puya* from the Andes of Bolivia. Journal of Bromeliad Society 43:211–215.

Morato, S. A., F. L. Franco, and E. J. Sanches. 2003. Uma nova especie de *Clelia* (Serpentes, Colubridae) do sul do Brasil. Phyllomedusa 2:93–100.

Scrocchi, G., and M. Viñas. 1990. El género *Clelia* (Serpentes, Colubridae) en la Republica Argentina: revisión y comentarios. Bolletino dei Musei Regionale Sciencia naturali di Torino 8: 487–499.

Zaher, H. 1996. A new genus and species of pseudoboine snake with a revision of the genus *Clelia* (Serpentes, Xenodontinae). Bolletino dei Musei Regionale Sciencia naturali di Torino 14:289–337.

Zaher, H. 1999. Hemipenial morphology of the South American xenodontine snakes, with a proposal for monophyletic Xenodontinae and a reapraisal of colubroid hemipenes. Bulletin of the American Museum of Natural History 240:1–168.

Accepted: 10 April 2005.