PROCEEDINGS OF THE 27th INTERNATIONAL HERPETOLOGICAL SYMPOSIUM on CAPTIVE PROPAGATION and HUSBANDRY

Hosted by
East Texas Herpetological Society and The Houston Zoo
Houston, Texas

September 3rd - September 6th, 2003

Welcome to the 27th Meeting of the International Herpetological Symposium

IHS Officers

President
WILLIAM BECKER
4054 Victoria Ct.
Columbia, MO 65201
(573) 874-3818

Vice President
JEFF ETTLING
Curator of Reptiles, Amphibians and Fish
St. Louis Zoo
(314) 781-0900 X364

IHS Secretary
STAN DRAPER
P.O. Box 16444
Salt Lake City, UT 84116

Treasurer
JOSEPH P. MAREK, JR.
10411 Balsamwood Ct.
Laurel, MD 20708
(410) 792-9840

IHS 2003 Program Coordinator
PAUL FREED
Herpetology Department
Houston Zoo
(713) 533-6655

ETHS/IHS Host Committee Chairman
RUSSELL WALKER
Trinity, TX
(936) 594-5554

IHS Advisors

JEFF BARRINGER
KEN FOOSE
PHILLIP SAMUELSON
BRENT SPENCER
RANDAL BERRY
VAL HORNYAK
VINCE SCHEIDT
PETER STRIMPLE
GIOVANNI FAGIOLI
DAVID HULMES
ANDREW SNIDER

International Herpetological Symposium, Inc.
27th Annual Meeting

IHS Officers

President
William Becker
4054 Victoria Court
Columbia, MO 65201
573.874.3818

Vice President
Jeff Ettling
Curator of Reptiles, Amphibians, and Fish
St. Louis Zoo
314.781.0900 ext. 364

IHS Secretary
Stan Draper
P. O. Box 16444
Salt Lake City, UT 84116

Treasurer
Joseph P. Marek, Jr.
10411 Balsamwood Court
Laurel, MD 20708
410.792.9840

IHS 2003 Program Coordinator
Paul Freed
Herpetology Department
Houston Zoo
713.533.6655

ETHS / IHS Host Committee Chairman
Russ Walker
Trinity, TX
936.594.5554

IHS Advisors

Vince Scheidt
Val Hornyak
Phillip Samuelson
Giovanni Fagioli
Ken Foose
David Hulmes
Andrew Snider
Brent Spencer
Jeff Barringer
Randal Berry
Peter Strimple

International Herpetological Symposium

27th Annual Meeting

Hosted by

The Houston Zoo and
The East Texas Herpetological Society
Houston, Texas USA

September 3 - September 6, 2003

Program and Abstracts

INTERNATIONAL HERPETOLOGICAL SYMPOSIUM
27TH ANNUAL MEETING

PROGRAM

Wednesday, September 3rd

5:00 - 8:00 p.m.	Registration – Crowne Plaza Resort
7:00 p.m. - ?	Ice Breaker – Hospitality Room

Thursday, September 4th

8:30 a.m. - 3:30 p.m.	Open Registration – Crowne Plaza Resort
9:00 - 9:15 a.m.	Open Remarks and Introductions Houston Zoo Director – **Rick Barongi**
9:15 - 10:00 a.m.	Keynote Presentation: **Bill Love** New Caledonia's Gecko Grandeur
10:00 - 10:45 a.m.	**Harold K. Voris** Evolution Across Life Zones: Reptiles Invade the Sea
10:45 - 11:00 a.m.	*Morning Break*
11:00 - 11:45 a.m.	**Rick Hudson** A Comprehensive Response to the Global Turtle Crisis
11:45 - 12:30 p.m.	**Jim Stout** Madagascar Canopy Wildlife
12:30 – 1:45 p.m.	*Lunch Break*
1:45 - 2:30 p.m.	**Eric Haug** Captive…Wild..What's the Real Issue? Assume Nothing!
2:30 - 3:15 p.m.	**John Pérez** A Review of an Internet Database of Venoms Found in the United States
3:15 – 3:30 p.m.	*Afternoon Break*
3:30 – 4:15 p.m.	**Dr. Mark Peckham** A Day in the Life of a Reptile Veterinarian

4:15 – 5:00 p.m.	**WORKSHOP** Open Forum "Ask the Vets" **Drs. David Doherty, Shirley Llizo, Mark Peckham, and Ed Wozniak**

Friday, September 5th

9:00 a.m. - 3:30 p.m.	Open Registration – Crowne Plaza Resort
9:00 - 9:45 a.m.	**Kathryn Vaughn** Natural History and Taxonomic Status of Mexican and Southwestern United States Blind Snakes Allied with *Leptotyphlops dulcis*
9:45 – 10:30 a.m.	**Patti Shoemaker** The Natural History of the Alligator Snapping Turtle
10:30 - 10:45 a.m.	*Morning Break*
10:45 - 11:30 a.m.	**Dante Fenolio** The Herpetofauna Exploiting Termite Mounds of the Upper Tocantins River Valley
11:30 – 12:15 p.m.	**Andy Price** The Houston Toad: A Case Study in Conservation Biology And Realpolitik
12:15 – 1:30 p.m.	*Lunch Break*
1:30 - 2:15 p.m.	**David Lazcano** The Effects of Habitat Burning on Mexican Herpetofauna
2:15 - 3:00 p.m.	**Kathy Love** A Cornucopia of Corn Snakes
3:00 - 3:15 p.m.	*Afternoon Break*
3:15 - 4:00 p.m.	**Paul Freed** The Herpetofauna of Houston
5:00 p.m.	Shuttle Buses leave for **Field Trip to the Houston Zoo**
6:00-8:00 p.m.	Herp House Tour/Bar-b-que
8:00 p.m.- ???	Millionaire Herp Game – Houston Zoo Auditorium

Saturday, September 6th

9:00 a.m. - 12:00 p.m.	Open Registration
9:00 - 9:45 a.m.	**John Malone** The Sexual Adventures of the Tropical Treefrogs of the Genus *Smilisca*
9:45 - 10:30 a.m.	**Jim O'Reilly** Caecilian Natural History
10:30 - 10:45 a.m.	*Morning Break*
10:45 – 11:30 a.m.	**Daniel Bennett** Gray's Monitor Lizard
11:30 – 12:15 p.m.	**Ed Wozniak** Venom-derived Disintegrins: A New Generation of Anti-tumor Peptides with Potential Therapeutic Value
12:15 – 1:30 p.m.	*Lunch Break*
1:30 – 2:15 p.m.	**Mahmood Sasa** The Status and Conservation of Crocodilians in Costa Rica: An Upgrade
2:15 – 3:00 p.m.	**William Lamar** Herping the Amazon in Peru
3:00 – 3:15 p.m.	*Afternoon Break*
3:15 – 4:00 p.m.	**Katie Hampson** Forest Fragmentation and Effects on Amphibian Communities in the Philippines
6:30 p.m. - ?	Banquet Dinner – Crowne Plaza Ballrom
	Banquet Speaker – **Jonathan A. Campbell** Neotropical Herpetofauna
	Presentation of the Joseph Laszlo Memorial Award
	Auction - Proceeds benefit next year's IHS! (Credit Cards Accepted)
Sunday, September 7th	11:00 a.m.- 6:00 p.m. **13th Annual East Texas Herpetological Society Breeder Expo at the Crowne Plaza Resort**

CONTENTS

Abstracts 1

A Review of an Internet Database
of Venoms Found in North America 11

ABSTRACTS

DANIEL BENNETT
Chapman Conservation
110 Sheffield Road
Glossop, SK13 8QU. UK.

Gray's Monitor Lizard

Monitor lizards usually eat animals, but two species in the Philippines have evolved into specialized fruit eaters. No other living reptile depends on fruit to the extent of these lizards and few vertebrates that live on land lack wings. A 20lb lizard that feeds on a very narrow range of fruits within a very complex and diverse forest structure seems an almost certain candidate for extinction, especially if its flesh has a reputation for being particularly delicious. The extinction process can be observed on Polillo Island where Gray's monitor lizard, *Varanus olivaceus*, is being extirpated from forest fragments as their food and shelter trees are destroyed. At current rates of logging the lizard will disappear from the area within about 50 years.

This project began in 1999 and is ongoing, currently supported by a number of US zoos. Learning about the habitat requirements of this lizard is difficult because they are notoriously shy and spend all but 40 minutes per week in tree canopies 20-40m above the ground. The only previous study of the species involved methods that are not appropriate today and so non destructive techniques were developed to investigate population status and structure, movement, habitat, diet and feeding behavior. Results suggest that the lizards respond to habitat degradation in a number of ways before eventually being forced out by lack of resources. In this presentation I will give an overview of the project and suggest some practical strategies for conservation of the animal and its habitat.

DANTÉ FENOLIO
Department of Zoology
University of Oklahoma

The Herpetofauna Exploiting Termite Mounds of the Upper Tocantins River Valley

The second largest ecosystem in Brazil's vast expanse is a poorly studied xeric habitat known as cerrado. Made of a mosaic of gallery forest, granitic outcroppings, palm clusters, and open grasslands, cerrado has surprised biologists with its biodiversity, including that of its herpetofauna. Until recently, research emphasis in Brazil had been placed on the last few patches of Atlantic forest and the Amazon; cerrado had taken a back seat and had been labeled as "wasteland." Contemporary efforts to study and document the environment have taken a feverish pace in light of its murky future. The unique biome has suffered an unprecedented rate of habitat alteration; over 60% has been seriously altered or destroyed in the last decade alone. Of the mechanisms employed by many cerrado organisms to withstand scorching day time temperatures, chilly night time temperatures, massive temperature fluctuations within a 24 hr. cycle (up to a 45° C swing), extensive wildfires, dehydrating winds, and long dry seasons is the inhabitance of termite mounds. Since 1995, over 1,500 termite mounds have been examined from the Upper Tocantins River Valley. Of 39 amphibian species documented from a site known as "Serra Da Mesa," 19 (49%) were found to inhabit termite

mounds. Of 75 reptile species from the same locality, 27 (36%) were found to do the same. Ongoing research may shed light on the specific advantages gained through the exploitation of the resource.

It is my contention, that with the development of the captive breeding of appropriate pet trade reptiles, we could be on the verge of another growth spurt in the reptile pet industry.

PAUL FREED
Herpetology Department
Houston Zoo
1513 N. MacGregor
Houston, Texas USA

The Herpetofauna of Houston

With approximately 90 varieties of herpetofauna in Harris County (45% of the 200 species found in Texas), Houston is home to one of the largest diversities of herps in the United States. Located in southeast Texas, the Houston area has several ideal herpetological habitats, including prairie grasslands, oak and pine forests, freshwater marshes, and savannahs to name but a few. As one of the largest cities in the United States, Houston encompasses a truly enormous piece of land, consisting of nearly 2,000 square miles, and it's not surprising that several of its amphibians and reptiles can even be found within its urban downtown areas.

KATIE HAMPSON
Department of Ecology and Evolutionary Biology
Princeton University
Princeton, New Jersey 08544 USA
E-mail: khampson@Princeton.EDU

Forest Fragmentation and Effects on Amphibian Communities in the Philippines

Collecting accurate information about the relative abundance and density of amphibians in rainforests communities presents considerable difficulties because they occupy a wide range of microhabitats in a very complex environment. Some species congregate in large numbers or are otherwise conspicuous but others are rare, cryptic or widely dispersed, sometimes so difficult to find they are completely overlooked. Data sets that are even remotely comparable for all species in the community are scarce and as a result it has been difficult to describe the ways communities change when habitat is destroyed or fragmented. Here I describe methods used to sample amphibian communities in Coorg (India) and Polillo (Philippines) and demonstrate an important edge effect in the latter, whereby forest specialists are eventually exterminated by increasing fragmentation.

"The Ultimate Reptile Store"
6 S #1400
77083 USA
4849

Wild . . . What's the Real Issue? Assume Nothing!

25 years experience in herp retail sales, I have had the opportunity to work with a tremen- of amphibians and reptiles and I've been able to observe first-hand which species have fared under captive conditions. There are two major factors in this type of work; understanding and natural history of the herptile and understanding the needs and capabilities of the customer. the broad range requirements of both entities, it is often a difficult and uphill battle to satisfy all It is a given that not all herp species will be suitable or even appropriate for all clients. Therefore, that a screening process be adopted that will best suit a chosen pet to its prospective owner. will highlight some of the more unusual, humorous, as well as serious situations that I have over the past quarter century.

HUDSON
Biologist
Zoo
Parkway
Texas 76110 USA
7555

Response to the Global Turtle Crisis

and tortoises are in trouble globally but nowhere is the situation more publicized than in Southeast The past ten years have seen the advent of a huge commercial market for chelonians to supply the food of southern China. Described as one of the greatest wildlife catastrophes of modern times, the Asian is unprecedented in it enormity and its risk. Measured in tens of tons of turtles per day, the clearly unsustainable and the far - reaching impacts are predictably dire. Not restricted to this the tentacles of this network are now extending to other regions including Madagascar, Africa and the Though the situation is most acute in Southeast Asia, populations globally are undergoing rapid de- well due to the activities of man.

of this magnitude demands a global response. This presentation will highlight some of the that has been made in recent years to address this crisis, focusing on some of the organizations that emerged to take action, primarily the Turtle Survival Alliance (TSA) and the Turtle Conservation Fund. of the myriad conservation actions being utilized including CITES regulation and enforcement, workshops, confiscations, range-country rescue centers, captive assurance colonies, capacity build- countries, and conservation biology research will be described.

WILLIAM W. LAMAR
College of Sciences & Mathematics
The University of Texas at Tyler
3900University Blvd
Tyler, Texas 75799 USA

Herping the Amazon in Peru

Want to get an idea what it is like to work in the world's largest rainforest with the world's most diverse herpetofauna? With nearly 400 species in an area smaller than east Texas, this is as exciting as it gets. If you can't experience it first-hand, this is the next best thing.

DR. DAVID LAZCANO
Universidad Autonoma de Nuevo Leon
Facultad de Ciencias Biologicas, Laboratorio de Herpetologia
Apartado Postal – 513, San Nicolas de los Garza, Nuevo Leon, C.P.66450 Mexico
Ph: (81) 8376-27-875, Lab: (81) 8332-2889, E-mail: dvlazczno@hotmail.com

The Effects of Habitat Burning on Mexican Herpetofauna

(No abstract submitted)

BILL LOVE
Blue Chameleon Ventures
P.O. Box 643
Alva, Florida 33920 USA
Ph: 239-728-2390
E-mail: bill@bluechameleon.org
www.bluechameleon.org

New Caledonia's Gecko Grandeur

The islands of New Caledonia have been isolated for eons, allowing a unique herpetofauna to evolve free of competition from virtually all forms of terrestrial predators. Only a few families of herps ever made it there to colonize the isolated islands. Subsequently, those couple groups - the geckos and the skinks - radiated out to include the largest members of the endemic terrestrial vertebrate fauna.

Bill Love was the expedition photographer on a biological survey of the small islands of southern New Caledonia with Philippe & Gigi de Vosjoli and Frank Fast for three weeks in 1997. His slide program will follow the group's explorations over the islands, focusing on the many species of animals encountered in the field, their macro and microhabitats, and other natural history observations concerning food, shelters, activity periods, etc. He will make you feel like you were there as he familiarizes you with all aspects of his voyage to a remote land harboring herps very popular in the pet trade today, but that few hobbyists will probably ever visit in person.

KATHY LOVE
CornUtopia
P.O. Box 643
Alva, Florida 33920 USA
Ph: (239) 728-2390
Email: kathy@cornutopia.com
www.corn-utopia.com

A Cornucopia of Corn Snakes

Over the past several decades, corn snakes have risen to claim the title of most commonly kept pet snakes in the world. The many qualities that have contributed to their immense popularity will be examined to demonstrate how they reached that status. The extremes of the current variation in color and patterns will also be shown. A brief history tracing the corn snake's evolution since the late 1950s (when the first 'albino' was found) will be presented as a prime example of a quintessential success story of modern herpetoculture.

Kathy Love has been keeping and breeding *Elaphe guttata* for over a quarter of a century. She is responsible for creating and refining some of the many morphs that have fueled their prevalence in the market. Her continuous work to improve husbandry and breeding techniques, and her renowned efforts of sharing her knowledge with all who have sought her help, have made her a major contributor to corn snakes' dominance of the pet snake trade today.

JOHN MALONE
Department of Biology
University of Texas – Arlington
Box 19498
Arlington, Texas 76019 USA

The Sexual Adventures of the Tropical Treefrogs of the Genus *Smilisca*

Two species of *Smilisca* place eggs in basins while the other four species lay eggs as a surface film in ephemeral pools. I tested two hypotheses concerning the evolutionary function of depositing eggs in basins. The microenviornment modification hypothesis targets potential abiotic benefits of depositing eggs in basins which may lead to increased metamorphic rates. The predator avoidance hypothesis predicts that eggs deposited in basins are segregated from predators and thus have increased survivorship. Results from field observations and egg palatability experiments support a predator avoidance hypothesis but also reveal tradeoffs from conspecific tadpoles and invertebrate predators that colonizebasins.

To understand the origin and distribution of the basin construction reproductive mode, I constructed a phylogeny of *Smilisca* using mtDNA data. These data produced a novel phylogenetic hypothesis which shows that the basin construction reproductive mode is constrained to a monophyletic unit composed of *S. sila* and *S. sordida*.

JAMES C. O'REILLY
Department of Biology
University of Miami
1301 Memorial Drive, 202 Cox Science Building
Coral Gables, Florida 33124-0421 USA

Caecilian Natural History
(No abstract submitted)

DR. MARK PECKHAM
Bellaire Boulevard Animal Clinic
6213 Bellaire Blvd.
Houston, Texas 77081 USA
Ph:(713) 772-5574

A Day in the Life of a Reptile Veterinarian

Dr. Peckhem will share some of experiences as a reptile veterinarian. Many topics will be discussed; including how to find a reptile veterinarian, quarantine basics, selected dreaded diseases, and case reports.

JOHN C. PÉREZ and ELDA E. SÁNCHEZ
Natural Toxins Research Center (NTRC)
Texas A&M University-Kingsville
Campus Box 158
Kingsville, TX 78363 USA

A Review of an Internet Database of Venoms Found in the United States

Snake venoms are complex mixtures of pharmacologically important molecules, some of which may have potential therapeutic agents. Snake venoms are rich, stable sources of disintegrins, metalloproteases, and fibrinolytic enzymes. The literature contains many references on how venom components are being used in medicine. Within the United States, there are 27 recognized species of venomous snakes. Despite this rather vast diversity of venomous snakes in North America, most of the biomedical research is conducted on a limited number of the more common venoms (*Crotalus atrox, C. adamanteus, C. viridis viridis, Agkistrodon contortrix contortrix, A. contortrix laticinctus*, and *A. piscivorus piscivorus*). The venoms from many of the North American species are not available for research. Therefore, the composition and potential usefulness in medicine has not been explored. The Natural Toxins Research Center (NTRC) serpentarium presently houses over 400 venomous snakes composed of 25 species and 33 subspecies. These snake venoms are cataloged in an Internet database along with their geographical location, sex, size, biological activities, high performance liquid chromatography profiles and electrophoretic titration profiles. These venoms can be queried through an on-line search routine. The objectives of the NTRC are to: 1) provide reliable information about venoms and related products from a wide variety of venomous snakes, 2) characterize venoms, and 3) develop a database which will be useful to investigators worldwide. Most of the snake venoms in the database are from the North American continent and all the venoms tested thus far are complex mixtures of many different toxins. The database will be expanded to include those antivenoms used to neutralize specific venoms. This information will be useful to the practitioner, herpetologist, poisonous control center technicians and forensic scientists.

ANDREW H. PRICE, Ph.D.
Natural Resources Scientist
Wildlife Diversity Program
Texas Parks and Wildlife Department
4200 Smith School Road
Austin, Texas 78744 USA
Ph:(512) 912-7022 Fax:(512) 912-7058

The Houston Toad: A Case Study in Conservation Biology and Realpolitik

The Houston Toad (*Bufo houstonensis*), a small member of the *Bufo americanus* species group endemic to east-central Texas, has resided longer on state, national, and international endangered species lists than most other taxa. This species disappeared from most of its historically know range following its discovery in the Houston, Texas, area following World War II, and was known from only a single locality during the three decades prior to 1990. Intensive surveys by Texas Parks and Wildlife Department personnel and associates have resulted in the discovery of new populations in 9 counties in central Texas. This species is restricted to deep sandy soils supporting ephemeral wetlands within native post oak/loblolly pine woodlands and savannas that have been subjected to minimal landscape-scale disturbance. Except for 2 sites, nothing is known about the demographics of the newly-discovered populations; the most robust population known still resides in Bastrop County. A total of 2833 adult Houston Toads have been marked with PIT tags in one watershed within Bastrop State Park from 1990-2002. Breeding choruses of up to 200 individuals form under specific climatic conditions over 1-4 nights during February and early March, separated by intervals of several days to several weeks. Individual females are rarely recaptured during the same breeding season, whereas males have been recaptured as often as 22 times in one year. Maximum longevity appears to be 6 years for males and 5 years for females. Individual toads have been recorded traveling distances of up to 1.3 km during the breeding season. Regional climatic regimes have a profound effect upon Houston Toad recruitment and survivorship, compounded by the current fragmented status of the species' populations. The conservation implications of these data and the contentious history of ongoing community efforts to implement a Habitat Conservation Plan for the Houston Toad in Bastrop County under the U.S. Endangered Species Act will be discussed.

MAHMOOD SASA
Instituto Clodomiro Picado
Universidad de Costa Rica
San Jose, Costa Rica.
email:sasa@cro.ots.ac.cr

The Status and Conservation of Crocodilians in Costa Rica: An Upgrade

Two species of crocodilians occur in Costa Rica, sharing wetlands, rivers, and marshes: the American crocodile *Crocodylus acutus*, and the spectacled caiman *Caiman crocodilus*. Both species are sympatric in the country, but microhabitat partition is evident in most localities along their distribution. Despite the long history of herpetological research in Costa Rica, it was not until recent that studies about the captive management, population ecology and behavior have been conducted in the country. In general, both species are common throughout their distribution in the country. Pacific populations of the American crocodile seem to be denser in Costa Rican than in other countries, probably reflecting conservation efforts that began late

1970's. In the Caribbean coast, most studies have focused in the spectacle caiman. Recent interests on the conservation biology and management of crocodilians have produced a national association of professionals, interested in the study of natural populations and management in captivity. In conjunction with colleagues from USA, an extensive campaign to demystify crocodilians has been in place. Nevertheless, recent reports reveal that conservation status of these populations is in jeopardy, especially in the north part of the country. Future research lines and conservation efforts are addressed here.

PATTI SHOEMAKER
Supervisor, Children's Zoo
Houston Zoo
1513 N. MacGregor
Houston, Texas 77030 USA
Ph :(713) 533-6500

The Natural History of the Alligator Snapping Turtle

Long a subject of folklore and southern culture, the Alligator Snapping Turtle, *Macroclemys temminckii*, with its worm-like lure, still fascinates young and young at heart herpetologists alike. Alligator Snapping Turtles are most common in the southeastern United States, from southern Georgia and northern Florida west to Texas and Oklahoma. They can even reach as far north as Illinois, Indiana and Kansas. The habitat of Alligator Snapping Turtles is rivers, lakes, ponds, swamps and bayous of the river systems of the Gulf of Mexico. Not much is known about the behavior and movements of America's largest freshwater turtle, but one thing is clear, the numbers of Alligator Snapping Turtles is declining at an unsustainable rate.

JIM STOUT
Herpetarium Supervisor
Oklahoma City Zoo
2101 NE 50th Street
Oklahoma City, Oklahoma 73111 USA
Ph: (405) 424-3344

Madagascar Canopy Wildlife

In October 2001 the French run "Canopy Raft Program" gave us the opportunity to explore one of the last remaining, primary rain forest in Madagascar. On the Masoala Peninsula we examined the herpetofaunal community with vertical and horizontal transects. We searched in emergent, upper, mid, lower and ground level habitats. Special attention was paid to phytotelmata and the fauna associated within. The Raft Program offered an assortment of unique collecting methods which will also be discussed.

R. KATHRYN VAUGHN, Ph.D. and
JAMES R. DIXON
Curator
Reptiles and Amphibians
Texas Cooperative Wildlife Collection
Texas A&M University
College Station, Texas 77843-2258 USA

Natural history and taxonomic status of Mexican and southwestern United States blind snakes allied with *Leptotyphlops dulcis*

The tiny, burrowing blind snakes of the genus *Leptotyphlops* are represented in the Americas by two species. One of these species, *Leptotyphlops dulcis* is distributed across the southwestern United States and the eastern half of Mexico. Aspects of the natural history of these secretive snakes will be discussed. In addition, the taxonomic status of *Leptotyphlops dulcis dulcis*, *L. dulcis dissectus*, and *L. dulcis myopicus* have been reexamined and updated, and these results will be presented.

HAROLD K. VORIS
Division of Amphibians and Reptiles
Field Museum of Natural History
Chicago, Illinois 60605 USA

Evolution Across Life Zones: Reptiles Invade the Sea

Among vertebrate historians, amphibians are known for colonizing the land while reptiles are renowned for completing the transition to a fully terrestrial way of life. Several key physiological and morphological innovations such as a leathery egg, and water-conserving kidneys and integument, made the latter transition possible. Since achieving this great evolutionary milestone many reptile lineages have experimented with a return to the sea. Why has this happened? A comparison of the various evolutionary experiments and an exploration of the common trends reveal that exploitation of a new food source seems to be a common factor and perhaps a driving force in the reptiles' return to an aquatic environment. This comparison also reveals that the return to the sea has been accompanied—not by reversals to previous conditions—but by novel adaptations that accomplish the old functions, as exemplified by the variety of specializations among the sea snakes. If abundant nutritional resources have been an important draw, what has limited these evolutionary reversals? A working hypothesis involving predation is explored to begin to try to answer this question.

EDWARD J. WOZNIAK D.V.M., Ph.D.

Venom-derived Disintegrins: A New Generation of Anti-tumor Peptides with Potential Therapeutic Value

Disintegrins are a class of non-toxic venom-derived peptides that are potent inhibitors of platelet aggregation. The mechanism of platelet aggregation inhibition is brought about by a complex cascade of intracellular reactions that are initiated by disintegrin engagement of integrin receptors on the platelet surfaces. Through their interaction with the integrins on tumor cells, disintegrins have recently been shown

to exhibit anti-cancer activities. Some disintegrins have been shown to arrest tumor growth and prevent metastasis by inhibiting cell proliferation, blocking cell motility, arresting vasculogenesis, and inducing endothelial cell apoptosis in human neoplasms. The net effect of these changes is a smaller, more localized tumor that is less likely to metastasize. Because of their therapeutic potential in the treatment of some cancers, there is biotechnological interest in developing some disintegrins as anti-neoplastic drugs.

While numerous disintegrins have been isolated from Asian Crotalinae, with the exception of the southern copperhead from which the anti-tumor peptide contortrostatin was derived, few disintegrin surveys have been done on New World species and no comparative studies have been done at the specific, subspecific and population levels of resolution (biotypes). With the rather extensive degree of geographic isolation and evolutionary divergence evident in the New World Crotalinae, the probability for unique disintegrins with anti-tumor properties in the vast array of known genera and species is predicted to be high. The purpose of this study was to characterize and compare the primary structure of disintegrins in a specified group of New World Crotalinae at the specific, subspecific and population levels of resolution. The specific aims were to test the following three hypotheses: 1) The structural features of venom-derived disintegrins vary between species and subspecies of the New World *Agkistrodon* species complex, *Crotalus lepidus and Atropoides nummifer nummifer*, 2) Structurally different disintegrins will have different integrin affinities and biological activities, 3) Some members of the New World Crotalinae undergo ontogenetic shifts in disintegrin expression.

Venom samples from each age-specific snake biotype were collected in chilled receptacles, centrifuged, sterile filtered and snap frozen at -70 C. Aliquots of raw venom were analyzed by polyacrylamide gel electrophoresis and the disintegrin fractions identified by Western blot using rabbit anti-contortrostatin antibodies. Fractions with proteolytic activity were identified by zymography. Initial analysis of the structural features of each disintegrin was accomplished by characterizing the corresponding mRNA sequences by high fidelity RT-PCR using custom designed oligonucleotide primers. Each resulting cDNA construct was inserted into a plasmid and cloned in competent JM-109 *E. coli* from which it will be recovered for sequence analysis. The amino acid sequences deduced from the cloned cDNA sequence information will be used in the initial comparison of primary structures.

Western blot analysis demonstrated similarly sized disintegrin-positive proteins (45-55 kDa and 13-16 kDa) in the venoms from all of the copperhead and cottonmouth subspecies in the study population. Both *A. bilineatus bilineatus* and *A. taylori* had disintegrin fractions that were easily distinguishable (150 kDa and 14.5-15 kDa) from those in the copperheads and the western cottonmouth. All of the non-enzymatic disintegrin-positive fractions identified in *Agkistrodon* sp. venoms approximate dimeric variants in size. *Crotalus lepidus lepidus* venom was found to contain disintegrin-positive proteins approximately 55 kDa and 8 kDa. The 8 kDa band is approximately the size of a monomeric disintegrin. Zymographic analysis with casein and type-A gelatin demonstrated proteolytic activity in all of the high molecular weight disintegrin-positive fractions in each of the venoms sampled. Interestingly, no disintegrins that were reactive with anti-contortrostatin antibody were demonstrated in *A. nummifer nummifer* venom. This finding suggests that this family of proteins to be either structurally different from those in other North American and Mexican Crotalinae of lacking all together.

High fidelity RT-PCR demonstrated similar RNA sequences in the venom glands of all of the coralline species sampled including *A. nummifer nummifer*. All resulting cDNA sequences were approximately 175 bp in size which very closely approximates the predicted size of the product sequence in contortrostatin. Each resulting cDNA construct (N=10 thus far) was successfully incorporated into a plasmid and used to transform competent *E. coli*. The average transformation efficiency was with the protocol used in our laboratory was 96%. This is an ongoing study.

A Review of an Internet Database of Venoms Found in North America

John C. Pérez*[1], Elda E. Sánchez[1,2], Jacob A. Galán[1], Angelica M. García[1], Gonzalo López Jr.[1], Alexis Rodríguez-Acosta[2]

[1]Natural Toxins Research Center (NTRC), Texas A&M University-Kingsville, MSC 158, Kingsville, TX 78363
[2]Universidad Central de Venezuela, Instituto de Medicina Tropical, Sección de Inmunoquímica, Apartado 47423, Caracas 1041, Venezuela

*Corresponding author: Dr. John C. Pérez, Tel.: (361) 593-3805; Fax: (361) 593-3798; E-mail address: jcp00@tamuk.edu

Abstract

The mission of the Natural Toxins Research Center (NTRC) is to provide global research, training, and resources that will lead to the discovery of medically important toxins found in snake venoms. The NTRC maintains a serpentarium that houses over 400 venomous snakes. An Internet database has been established that allows the user to view the snakes and their geographic distribution, venom profiles, and enzymatic activities of venoms found in North America. As the venoms are, collected, purified and characterized, the NTRC will also be an important resource for testing and developing antivenoms. Two antivenoms, Antivipmyn, (Fab_2H) and CroFab, (FabO), were compared and both were effective in neutralizing New World pit viper venoms.

Introduction

The Natural Toxins Research Center (NTRC) has a serpentarium that presently houses over 400 venomous snakes found in North America. These snakes are cataloged and the information is entered into an Internet database. The venoms are characterized by high performance liquid chromatography (HPLC), electrophoretic titration (ET) profiles, and enzyme activities, and the information can be queried through an on-line search routine. The database is useful to investigators interested in isolating enzymes, toxins and disintegrins in snake venoms (Perez et al., 2001). In addition, the NTRC has the venomous snakes, animal facility, in vivo and in vitro assay for testing the efficacy of antivenoms.

Sanchez et. al., (2003) tested the efficacy of Fab_2H produced in Mexico by Bioclon, and FabO that is currently used in the United States with15 snakes found in the United States. Three different assays were used to test the efficacy of the antivenoms, the *in vivo* serum protection test (the effective dose that will protect 50% of a mouse population, ED_{50}), antihemorrhagic and anticoagulant assays. Both antivenoms were effective in neutralizing the lethal dose that kills 50% of a mouse population (LD_{50}) of the North American pit viper venoms; however, Fab_2H was more effective in neutralizing the hemorrhagic activities of the venoms. The snakes selected were commonly reported to be responsible for bites; and eight of the 15 venoms reported were from species of snakes used in a study published by Consroe et al. (1995) to test the efficacy of FabO.

A physician has no assurance of the effectiveness of commercial antivenom since each snake venom even within the same species could be different. The composition of the particular venom causing the damage may be different from the venom used to produce the antivenom. All that can really be assumed is that polyvalent

antivenom could have a high probability of neutralizing the venom and should reduce the recovery time or decrease the death rate.

Many of the venomous snakes found in zoos and serpentariums in the United States are not North American pit vipers. It is generally not known if Antivipmyn (Fab_2H) or CroFab (FabO) can neutralize venoms found those facilities.

The purpose of this paper is to show the usefulness of the NTRC database in biomedical research and give a specific example of how the resources at the NTRC can be used for determining the efficacy antivenoms. The effectiveness of Antivipmyn (Fab_2H) in the neutralization of hemorrhagic activity and lethal properties of snake venoms of six New World pit vipers were reported for the first time (*Agkistrodon bilienatus*, *Bothrops atrox*, *B. asper*, *Crotalus durissus durissus*, *Crotalus durissus terrificus*, and *Lachesis muta*). In order to present more information about antivenoms, the literature on antivenoms that neutralize the venoms of snakes found in the United States was reviewed (Sánchez et. al., 2003). Two different assays were used to test the efficacy of Fab_2H; the ED_{50} and the antihemorrhagic assay.

Methods and Materials

United States Venoms

All venoms used in the Sánchez et. al., (2003) study were extracted from snakes maintained at the NTRC on the average of every six weeks with the exception of *Crotalus adamanteus* venom which was purchased from Sigma-Aldrich, Co. The snakes are allowed to bite into a parafilm membrane over a disposable container and the venom is immediately transferred to Eppendorff tubes. The venoms are centrifuged for 5 min at 23° C at 12,800 x g to remove cellular debris. They are then transferred to labeled vials and stored at -78° C until lyophilized. Venoms are lyophilized once they reach a volume of 1 mL and venoms of the same species are never mixed unless it is required.

In the Sánchez et. al., (2003) study, equal mixtures of lyophilized venoms of the same species from different locatins were pooled (Table 1). The lyophilized venom samples were reconstituted in physiological saline, centrifuged at 500 x g for 10 min and filtered using a Millipore Millix HV 0.45 µm filter unit prior to use. Many of the snake venoms have been previously characterized by high performance liquid chromatography (HPLC) and electrophoretic (ET) profiles and can be found on the Internet (Perez et al., 2001). The HPLC and ET profiles are useful in determining the difference amongst venoms. All protein determinations for venoms and antivenoms were measured at 280 nm.

The mouth and fang of an Eastern Coral Snake. Photo by Bill Love.

Latin American Venoms

Lyophilized venoms were obtained from various providers, since the NTRC currently houses only a few snakes from Latin America. The venoms of *Bothrops asper* and *Crotalus durissus durissus* were provided by the Instituto Bioclon/Laboratorio Silanes, S.A. de C.V., Calzada de Tlaipan No. 4687 Col. Toriello Guerra, 14050 Mexico, D.F. The venoms of *Lachesis muta* and *Agkistrodon bilineatus* were provided by Biotoxins Inc. Quality Venom Products, 6705 E. Bronson Memorial Highway, St. Cloud, FL 34771, USA. The venoms of *Bothrops atrox* and *Crotalus durissus terrificus* were obtained from the Kentucky Reptile Zoo, 200 L & E Railroad, Glade, KY 40376.

The lyophilized venom samples were reconstituted in physiological saline, centrifuged at 500 x g for 10 min and filtered using a Millipore Millix HV 0.45 µm filter unit prior to use. All protein determinations for venoms and antivenoms were measured at 280 nm in accordance to a bovine serum albumin (BSA) standard.

Antivenoms

Antivipmyn (Fab_2H) is a polyclonal antivenom $(Fab')_2$ fragment of equine origin produced by Instituto Bioclon in Mexico. The venoms used to produce the Fab_2H were that of *Crotalus durissus durissus* and *Bothrops asper.* The second antivenom is an affinity-purified Fab fragment of ovine origin (FabO) produced by Therapeutic Antibodies, Inc., London, England. The snake venoms used to produce FabO were *Crotalus atrox* (Western Diamondback Rattlesnake), *Crotalus adamanteus* (Eastern Diamondback Rattlesnake), *Crotalus scutulatus scutulatus* (Mojave Rattlesnake), and *Agkistrodon piscivorus piscivorus* (Eastern Cottonmouth).

Hemorrhagic Assay

The method of Omori-Satoh et al. (1972) was used to determine the minimal hemorrhagic dose (MHD) for the crude venoms. A series of eight dilutions were made for each snake venom, of which 0.1 mL of each dilution was injected intracutaneously into the depilated backs of rabbits. After 24 h, the rabbit was sacrificed and the skin removed. A caliper was used to measure the hemorrhagic diameter on the skin and the MHD determined. The MHD is defined as the amount of venom protein that causes a 10 mm hemorrhagic spot.

Antihemorrhagic assay

A modified method used by Gutiérrez et al. (1985) was followed. Four hundred microliters of crude venom containing 20 MHD were incubated for 1 h at 25° C with 400 µl of various concentrations of antivenom. This was done for each hemorrhagic venom. The backs of rabbits were depilated and 0.1 mL of each concentration of antivenom was injected intracutaneously. Concentrations of antivenom were selected which neutralized 50% of one MHD of the venoms. Separate rabbits were used for each venom. The antihemorrhagic dose (AHD) is defined as that concentration of antivenom that neutralizes 50% of one MHD.

Lethal Dose (LD_{50})

Six groups of eight mice (BALB/c) were used for each venom . Venoms were dissolved in physiological saline at the highest concentration of venoms that were used for injection. Serial dilutions of 1.2 fold using saline were made to obtain five additional concentrations. All solutions during the experiment were stored at 4° C and warmed to 37° C just before being injected into mice. The lethal toxicity was determined by injecting 0.2 mL of venom (at various concentrations) into the tail veins of 18-20 g female mice. The injections

were administered using a 1-mL syringe fitted with a 30-gauge, 0.5-inch needle. Saline (0.15M NaCl) controls were used. The endpoint of lethality of the mice was determined after 48 h. The calculations for the LD_{50} were generated by a program on the NTRC homepage (http://ntri.tamuk.edu/serp/index.html) which was based on the method developed by Reed and Muench, (1938).

Serum Protection Test (ED_0)

For each antivenom concentration, six groups of eight mice were challenged with a mixture of three LD_{50} of *venom. Six doses of antivenom were used at each level. Stock* venom solutions containing *30* LD_{50} were prepared and stored at 0° C before being used. For each group of mice, equal volumes of venom and antivenom were mixed and incubated at 37?C for 30 min. Each mouse was injected with 0.2 mL of venom/ antivenom mixture into their tail veins. The mice were observed for 48 hr and the percent survival and ED_{50} was calculated. Saline controls and antivenom controls were used. The calculations for the ED_{50} were generated by a program on the NTRC homepage (http://ntri.tamuk.edu/serp/index.html) which was based on the method developed by Reed and Muench, (1938).

Results

The NTRC database (http://ntri.tamuk.edu) is online and can be accessed by investigators worldwide. The database has over 400 venomous snakes from 27 species and can be queried by the user to search by scientific name, common names, sex or identification number (Avid #). Information about snake capture location, date and time collected, weight and length of snake, feeding and venom extracting records are available. The venoms with an asterisk following their names have additional information, such as a photo of the snake, map showing the geographical location, DEAE/HPLC profile, ET profile, and various enzyme activities of the venom fractions. The information in the database will be useful information to those producing antivenoms and be a source of venom used in the manufacturing of antivenoms.

The database was a useful for selecting venoms that were used in testing the effectiveness of antivenoms (Sánchez et. al., 2003). The minimal hemorrhagic doses for the 15 venoms ranged from 0.3 to 143 µg with the most hemorrhagic venom being *C. adamanteus*, and the least being *C. s. scutulatus* (Table 1). Fab_2H antivenom was able to neutralize the hemorrhagic activity of all the hemorrhagic venoms, while FabO was able to neutralize 11 out of the 14 hemorrhagic venoms (Table 1).

The intravenous (I.V.) LD_{50} for 15 venoms found in the United States ranged from 0.47 to 6.8 mg/kg body weight with *C. s. scutulatus* Type A being the most potent, and the least potent was *A. c. laticinctus* (Table 2). Fab_2H was effective in neutralizing the LD_{50} of all the venoms used in this study while FabO was effective in neutralizing all the venoms with the exception of *C. m. molossus* venom. The venoms that were neutralized by FabO had ED50 that had several magnitudes (2.1-6.7) better than Fab_2H (Table 2). In those cases in which Fab_2H neutralized better than FabO, Fab_2H was just 1.1-3 times better.

The minimal hemorrhagic doses for the six venoms not found in Latin America ranged from 1.17 to 312 µg with the most hemorrhagic venom being *B. atrox*, and the least being *C. d. terrificus* (Table 3). Fab_2H was able to neutralize the hemorrhagic activity of all the hemorrhagic venoms (Table 3). FabO was not used in this study since it could not be purchased. Fab_2H was most effective in neutralizing the hemorrhagic activity of *B. atrox* (5 µg), while the hemorrhagic activity of *C. d. durissus* was least effective in being neutralized (425 µg). The results follow similar patterns reported by Sánchez et. al., (2003). The most hemorrhagic venom (the minimal amount of protein required to produce a 10 mm hemorrhagic spot) required less Fab_2H to neutralize the hemorrhagic activity, and a less hemorrhagic venom required more Fab_2H for neutralization (Table 3).

The I.V. LD_{50} for six venoms found in Latin America ranged from 0.22 to 6.3 mg/kg body weight with *C. d.* *scutulatus* being the most potent and the least potent was *L. muta* (Table 4). Fab_2H was effective in neutralizing the LD_{50} of all the venoms with the exception of *L. muta*. The venom of *A. bilineatus* was neutralized with the least amount of Fab_2H, while the venom of *B. atrox* required 14 times more Fab_2H for an ED_{50}. The most toxic venoms required less Fab_2H and the least toxic venoms required more Fab_2H to neutralize the venom. This was consistent with the finding of Sánchez et. al., (2003). If snake venoms are extremely toxic, a high dilution is required to obtain an LD_{50}. The antibody to venom ratio would be much higher and therefore the most toxic venoms would be neutralized with less antivenom. A much lower dilution would be required for a LD_{50} of a less toxic venom. The antibody to venom ration would be lower and the least toxic venoms would be more difficult to neutralize.

Discussion

Sánchez et. al., (2003) reported that both Fab_2H and FabO, were effective in neutralizing the LD_{50} of 15 North American venoms; however, Fab_2H was most effective in neutralizing the hemorrhagic activity. Therefore, Fab_2H appears to be an alternate choice in the treatment of snakebite envenomations in the United States and Canada particularly when considering cost and availability.

Preliminary data show that Fab_2H neutralizes the hemorrhagic activity of six Latin American venoms. In the ED_{50} assay, the antivenom was effective in neutralizing 5 of the 6 venoms. *L. muta* was not neutralized in the ED_{50} assay under the conditions used in our study. Fab_2H appears to be a possible choice in the treatment of snakebite envenomations of exotic snakes found in zoos and serpentariums.

The Sánchez et. al., (2003) study and this study suggest that there is a need to change the way antivenom efficacy is measured. It would seem more appropriate to evaluate antivenom in a manner that is similar to the way a physician treats patients. A more meaningful test would be to inject a fixed amount of antivenom into a mouse and determine the LD_{50} in protected mice. This procedure would measure the effects of minor components in venom and would be closer to the way a physician treats snakebite victims.

The way in which the venoms are collected at the NTRC is an important resource to biomedical research. The serpentarium has over 400 venomous snakes from North America and the number will continue to grow until the maximum capacity of approximately 660 is reached. These snakes are pit tagged to insure identity of the venom. Collection data, along with venom characteristics are entered into an Internet database, which can be queried. The venom HPLC, ET, and enzymatic profiles of venoms can be reviewed from the Internet and this online information is useful to investigators worldwide (Perez et al., 2001). Since venoms are different from one location to another the venoms are never pooled and research can use the same venom from a snake in future research projects.

References Cited

Cousse P, Gerrish, K, Egen N., and Russell F.E. Grttish, D. C Smith, A Sidki. And Landon, J. T. 1995. Comparison of a new ovine antifen binding fragment (Fab) antivenin for United States Crotalidae with the commercial antivenin for protection against veno-induce lethality in mice. Am. J. Trop. Med.Hyg. 5341, 507-510.

Gutiérrez, J.M., Gené, J.A., Rojas, G., Cerdas, L., 1985. Neutralization of proteolytic and hemorrhagic activities of Costa Rican snake venoms by a polyvalent antivenom. Toxicon 23, 887-893.

Omori-Satoh, T., Sadahiro, S., Ohsaka, A., Murata, R., 1972. Purification and characterization of an antihemorrhagic factor in the serum of *Trimeresurus flavoviridis*, a crotalid. Biochim. Biophys. Acta 285,

414-426.

Perez, J.C., McKeller, M.R., Pérez, J.C., Sánchez, E.E., Ramírez, M.S. 2001.An internet database of crotaline venom found in the United States. Toxicon 39,621-632.

Reed, L.S., Muench, H., 1938. A sample method of estimating fifty percent end point. Am. J. Hyg. 27, 493-497.

Sánchez, E.E., Galán, J.A., Perez, J.C., Rodríguez-Acosta, A., Chase, P.B., Pérez, J.C. 2003. The efficacy of two antivenoms against the venom of North American snakes. Toxicon. 41,357-365.

World Health Organization (WHO). 1981. Progress in the characterization of venoms and Standardization of Antivenoms. WHO Offset Publication 58 Geneva.

Acknowledgements

This research was supported in part by the NIH/RIMI (2P20RR11594), NIH/SCORE (5S06GM08107-27), and the NIH/NCRR (1P40RR018300-01) grants. A special thanks to Nora Diaz De Leon, NTRC Administrative Officer, for her technical assistance.

Appendices

Table 1

Minimal Hemorrhagic Dose (MHD) for 15 snake venoms and the Antihemorrhagic Dose (AHD) of two Antivenoms (Sánchez et al., 2003).

Venom[a]	MHD[b] (µg)	Fab_2H AHD (µg)[c]	FabO AHD (µg)	Ratio[d]
Crotalus adamanteus[e]	0.3	1 (1)	4 (1)	0.25
C. viridis	0.7	4.4 (3)	4.4 (2)	1.0
C. helleri	2.25	3.3 (2)	13.3 (5)	0.25
Micrurus catenatus tergiminus	2.4	8.8 (4)	13.3 (5)	0.66
C. atrox	2.5	27 (7)	7 (4)	3.85
C. e. edwardsii	3.5	26.6 (6)	141.7 (8)	0.19
C. h. horridus	5.6	4.4(3)	6.5 (3)	0.67
C. s. scutulatus-B	12.2	283 (11)	35.4 (6)	7.9
C. m. molossus	12.5	35.4 (8)	283 (9)	0.12
A. p. leucostoma	29	70.8 (9)	141.7 (8)	0.49
C. h. atricaudatus	37.5	212 (10)	-	
C. o. oreganus	43	425 (12)	-	
A. c. laticinctus	67	283(11)	-	
A. c. contortrix	143	26.5 (5)	70.8 (7)	0.37
C. s. scutulatus-A	£			

[a]Pooled venom obtained fromthe NTRC serpentarium.
[b]MHD: The amount of venom protein injected into the back of a depilated rabbit causing a 10 mm hemorrhagic spot in diameter.
[c]Antivenoms were at a starting concentration of 8.5 mg/mL.
[d]AHD: Antihemorrhagic Dose-The amount of antivenom (µg) that neutralizes 50% of 1 MHD of venom.
The AHD is calculated by dividing the starting concentration of antivenom by the antihemorrhagic titer that neutralizes 50% of 1 MHD and then multiplying by the amount of volume injected into the back of a depilated rabbit.
[e]Fab_2H AHD/ FabO AHD.
[f]*C. adamanteus* venom was purchased from Sigma-Aldrich, Co.
[g]- indicates that the MHD was not neutralized with equal volume of antivenom at a concentration of 8.5 mg/mL
[h]Venom contains no hemorrhagic activity.
[i]Number in parenthesis indicates the rank order in which the antivenom neutralized the MHD.
Shaded area indicates the antivenom that requires less protein for neutralization.

Table 2

LD_{50} and ED_{50} of 15 snake venoms and two different antivenoms (Sánchez et al., 2003).

Venom[a]	LD_{50}[b]	Fab_2H ED_{50}[c]	FabO ED_{50}[c]	Ratio[d]
Crotalus s. scutulatus Type A	*0.47*	*140.5 (11)*	*21 (4)*	*6.7*
C. h. horridus	0.53	111.6 (8)	20.9 (3)	5.3
C. h. atricuadatus	1.26	58.9 (3)	8.9 (1)	6.6
C. v. viridis	1.56	93.6(7)	17.7 (2)	5.2
Sistrurus catenatus edwardsii	1.7	140 (10)	226 (12)	0.6
C. adamanteus[e]	1.84	34.9 (1)	70 (6)	0.50
C. v. helleri	1.9	46.7 (2)	70 (6)	0.67
C. v. oreganus	2.1	114.1 (9)	121 (10)	0.94
S. c. tergiminus	2.1	83.1(4)	78.4 (8)	1.05
A. p. leucostoma	2.75	186.8(12)	55.2 (5)	3.3
C. m. molossus	4.84	93.1 (6)	NP (15)	
C. atrox	5.1	295 (14)	310 (14)	0.95
C. s. scutulatus Type B	5.1	88.4 (5)	278 (13)	0.31
A. c. contortrix	5.2	331.6(15)	93.7 (9)	3.5
A. c. laticinctus	6.8	293(13)	140.5 (11)	2.1

[a]Pooled venom obtained from the NTRC serpentarium.

[b]The LD_{50} is the concentration of venom (mg/Kg body weight) required to kill 50% of the BALB/c mice injected iv with 0.2mL of the various snake venoms. LD_{50} was calculated using the LD_{50} calculator on the Natural Toxins Research Center's homepage at http://ntri.tamuk.edu/cgi-bin/ld_{50}/ ld_{50}.

[c]Expressed as mg of antivenom/kg of mouse body weight; ED_{50} values were determined against 3 x LD_{50}
of venoms.

[d]ED_{50} of Fab_2H antivenom/ED_{50} of the FabO antivenom.

()Number in parenthesis indicates the rank order in which the antivenom neutralized 3 x LD_{50}.. Shaded area indicates which antivenom required less protein for neutralization.

[e] *C. adamanteus* was purchased from Sigma-Aldrich, Co.

NP: Antivenom did not protect.

Table 3

Minimal Hemorrhagic Dose (MHD) for 6 snake venoms and the Antihemorrhagic Dose (AHD) of Antivipmyn Antivenom

Venom	MHD[d] (µg)	Fab_2H AHD (µg)[e]
Bothrops asper[a]	5.6	26.5
Crotalus durissus durissus[a]	70	425
Lachesis muta[b]	15	13.2
Agkistrodon bilineatus[b]	1.35	8.8
Bothrops atrox[c]	2.0	5
Crotalus durissus terrificus[c]	312	53

[a]Venom samples obtained from Instituto Bioclon/Laboratorio Silanes, S.A. de C.V.,Calzada de Tlalpan No. 4687
Col. Toriello Guerra, 14050 Mexico, D.F.
[b]Venom samples obtained from Biotoxins Inc. Quality Venom Products, 6705 E. Bronson Memorial Highway,
St. Cloud, FL 34771 U.S.A.
[c]Venom samples obtained from the Kentucky Reptile Zoo, 200 L & E Railroad, Glade, KY 40376
[d]MHD: The amount of venom protein injected into the back of a depilated rabbit causing a 10 mm hemorrhagic spot in diameter.
[e]Antivenoms were at a starting concentration of 8.5 mg/mL.
AHD: Antihemorrhagic Dose-The amount of antivenom (µg) that neutralizes 50% of 1 MHD of venom.
The AHD is calculated by dividing the starting concentration of antivenom by the antihemorrhagic titer that neutralizes 50% of 1 MHD and then multiplying by the amount of volume injected into the back of a depilated rabbit.

Table 4

LD_{50} and ED_{50} of 6 snake venoms and Antivipmyn Antivenom

Venom	LD_{50}d	Fab_2H ED_{50}e
Bothrops asper[a]	1.2	24.52
Crotalus durissus durissis[a]	3.6	93.63
Lachesis muta[b]	6.3	NP
Agkistrodon bilineatus[b]	1.16	17
Bothrops atrox[c]	4.9	117.4
Crotalus durissus terrificus[c]	0.2	224

[a]Venom obtained from Instituto Bioclon/Laboratorio Silanes, S.A. de C.V., Calzada de Tlalpan No. 4687, Col. Toriello Guerra, 14050 Mexico, D.F.

[b]Venom samples from Biotoxins Inc., Quality Venom Products , 6705 E. Bronson Memorial Highway, St. Cloud, FL 34771 U.S.A.

[c]Venom obtained from Kentucky Reptile Zoo, 200 L & E Railroad, Glade, KY 40376

[d]The LD_{50} is the concentration of venom (mg/Kg body weight) required to kill 50% of the BALB/c mice injected iv with 0.2mL of the various snake venoms. LD_{50} was calculated using the LD_{50} calculator on the Natural Toxins Research Center's homepage at http://ntri.tamuk.edu/cgi-bin/ld_{50}/ld_{50}.

[e]ED_{50} of Fab_2H antivenom/ED_{50} of the FabO antivenom expressed as mg of antivenom/kg of mouse body weight; ED_{50} values were determined against 3 x LD_{50} of venoms_

Abstracts

DANIEL BENNETT
Mampam Conservation
118 Sheffield Road
Glossop, SK13 8QU. UK.

Gray's Monitor Lizard

Monitor lizards usually eat animals, but two species in the Philippines have evolved into specialized fruit eaters. No other living reptile depends on fruit to the extent of these lizards and few vertebrates that live on fruit lack wings. A 20lb lizard that feeds on a very narrow range of fruits within a very complex and diverse forest structure seems an almost certain candidate for extinction, especially if its flesh has a reputation for being particularly delicious. The extinction process can be observed on Polillo Island where Gray's monitor lizard, *Varanus olivaceus*, is being extirpated from forest fragments as their food and shelter trees are destroyed. At current rates of logging the lizard will disappear from the area within about 50 years.

This project began in 1999 and is ongoing, currently supported by a number of US zoos. Learning about the habitat requirements of this lizard is difficult because they are notoriously shy and spend all but 40 minutes per week in tree canopies 20-40m above the ground. The only previous study of the species involved methods that are not appropriate today and so non destructive techniques were developed to investigate population status and structure, movement, habitat, diet and feeding behavior. Results suggest that the lizards respond to habitat degradation in a number of ways before eventually being forced out by lack of resources. In this presentation I will give an overview of the project and suggest some practical strategies for conservation of the animal and its habitat.

DANTÉ FENOLIO
Department of Zoology
University of Oklahoma

The Herpetofauna Exploiting Termite Mounds of the Upper Tocantins River Valley

The second largest ecosystem in Brazil's vast expanse is a poorly studied xeric habitat known as cerrado. Made of a mosaic of gallery forest, granitic outcroppings, palm clusters, and open grasslands, cerrado has surprised biologists with its biodiversity, including that of its herpetofauna. Until recently, research emphasis in Brazil had been placed on the last few patches of Atlantic forest and the Amazon; cerrado had taken a back seat and had been labeled as "wasteland." Contemporary efforts to study and document the environment have taken a feverish pace in light of its murky future. The unique biome has suffered an unprecedented rate of habitat alteration; over 60% has been seriously altered or destroyed in the last decade alone.
Of the mechanisms employed by many cerrado organisms to withstand scorching day time temperatures, chilly night time temperatures, massive temperature fluctuations within a 24 hr. cycle (up to a 45° C swing), expansive wildfires, dehydrating winds, and long dry seasons is the inhabitance of termite mounds. Since 1995, over 1,500 termite mounds have been examined from the Upper Tocantins River Valley. Of 39 amphibian species documented from a site known as "Serra Da Mesa," 19 (49%) were found to inhabit termite mounds. Of 75 reptile species from the same locality, 27 (36%) were found to do the same. Ongoing research may shed light on the specific advantages gained through the exploitation of the resource.

It is my contention, that with the development of the captive breeding of appropriate pet trade reptiles, we could be on the verge of another growth spurt in the reptile pet industry.

PAUL FREED
Herpetology Department
Houston Zoo
1513 N. MacGregor
Houston, Texas USA

The Herpetofauna of Houston

With approximately 90 varieties of herpetofauna in Harris County (45% of the 200 species found in Texas), Houston is home to one of the largest diversities of herps in the United States. Located in southeast Texas, the Houston area has several ideal herpetological habitats, including prairie grasslands, oak and pine forests, freshwater marshes, and savannahs to name but a few. As one of the largest cities in the United States, Houston encompasses a truly enormous piece of land, consisting of nearly 2,000 square miles, and it's not surprising that several of its amphibians and reptiles can even be found within its urban downtown areas.

KATIE HAMPSON
Department of Ecology and Evolutionary Biology
Princeton University
Princeton, New Jersey 08544 USA
E-mail: khampson@Princeton.EDU

Forest Fragmentation and Effects on Amphibian Communities in the Philippines

Collecting accurate information about the relative abundance and density of amphibians in rainforests communities presents considerable difficulties because they occupy a wide range of microhabitats in a very complex environment. Some species congregate in large numbers or are otherwise conspicuous but others are rare, cryptic or widely dispersed, sometimes so difficult to find they are completely overlooked. Data sets that are even remotely comparable for all species in the community are scarce and as a result it has been difficult to describe the ways communities change when habitat is destroyed or fragmented. Here I describe methods used to sample amphibian communities in Coorg (India) and Polillo (Philippines) and demonstrate an important edge effect in the latter, whereby forest specialists are eventually exterminated by increasing fragmentation.

ERIC HAUG
Pets-A-Plenty "The Ultimate Reptile Store"
6763 Highway 6 S #1400
Houston, Texas 77083 USA
Ph: (281) 568-4849

Captive...Wild...What's the Real Issue? Assume Nothing!

With more than 25 years experience in herp retail sales, I have had the opportunity to work with a tremendous diversity of amphibians and reptiles and I've been able to observe first-hand which species have fared better than others under captive conditions. There are two major factors in this type of work; understanding the biology and natural history of the herptile and understanding the needs and capabilities of the customer. Considering the broad range requirements of both entities, it is often a difficult and uphill battle to satisfy all involved. It is a given that not all herp species will be suitable or even appropriate for all clients. Therefore, it is incumbent that a screening process be adopted that will best suit a chosen pet to its prospective owner. This presentation will highlight some of the more unusual, humorous, as well as serious situations that I have dealt with over the past quarter century.

RICK HUDSON
Conservation Biologist
The Fort Worth Zoo
1989 Colonial Parkway
Fort Worth, Texas 76110 USA
(817) 759-7555

A Comprehensive Response to the Global Turtle Crisis

Turtles and tortoises are in trouble globally but nowhere is the situation more publicized than in Southeast Asia. The past ten years have seen the advent of a huge commercial market for chelonians to supply the food markets of southern China. Described as one of the greatest wildlife catastrophes of modern times, the Asian Turtle Crisis is unprecedented in it enormity and its risk. Measured in tens of tons of turtles per day, the trade is clearly unsustainable and the far - reaching impacts are predictably dire. Not restricted to this region, the tentacles of this network are now extending to other regions including Madagascar, Africa and the U.S. Though the situation is most acute in Southeast Asia, populations globally are undergoing rapid declines as well due to the activities of man.

A challenge of this magnitude demands a global response. This presentation will highlight some of the progress that has been made in recent years to address this crisis, focusing on some of the organizations that have emerged to take action, primarily the Turtle Survival Alliance (TSA) and the Turtle Conservation Fund. Examples of the myriad conservation actions being utilized including CITES regulation and enforcement, training workshops, confiscations, range-country rescue centers, captive assurance colonies, capacity building in range countries, and conservation biology research will be described.

WILLIAM W. LAMAR
College of Sciences & Mathematics
The University of Texas at Tyler
3900University Blvd
Tyler, Texas 75799 USA

Herping the Amazon in Peru

Want to get an idea what it is like to work in the world's largest rainforest with the world's most diverse herpetofauna? With nearly 400 species in an area smaller than east Texas, this is as exciting as it gets. If you can't experience it first-hand, this is the next best thing.

DR. DAVID LAZCANO
Universidad Autonoma de Nuevo Leon
Facultad de Ciencias Biologicas, Laboratorio de Herpetologia
Apartado Postal – 513, San Nicolas de los Garza, Nuevo Leon, C.P.66450 Mexico
Ph: (81) 8376-27-875, Lab: (81) 8332-2889, E-mail: dvlazczno@hotmail.com

The Effects of Habitat Burning on Mexican Herpetofauna

BILL LOVE
Blue Chameleon Ventures
P.O. Box 643
Alva, Florida 33920 USA
Ph: (239) 728-2390 E-mail: bill@bluechameleon.org
www.bluechameleon.org

New Caledonia's Gecko Grandeur

The islands of New Caledonia have been isolated for eons, allowing a unique herpetofauna to evolve free of competition from virtually all forms of terrestrial predators. Only a few families of herps ever made it there to colonize the isolated islands. Subsequently, those couple groups - the geckos and the skinks - radiated out to include the largest members of the endemic terrestrial vertebrate fauna.

Bill Love was the expedition photographer on a biological survey of the small islands of southern New Caledonia with Philippe & Gigi de Vosjoli and Frank Fast for three weeks in 1997. His slide program will follow the group's explorations over the islands, focusing on the many species of animals encountered in the field, their macro and microhabitats, and other natural history observations concerning food, shelters, activity periods, etc. He will make you feel like you were there as he familiarizes you with all aspects of his voyage to a remote land harboring herps very popular in the pet trade today, but that few hobbyists will probably ever visit in person.

KATHY LOVE
CornUtopia
P.O. Box 643
Alva, Florida 33920 USA
Ph: (239) 728-2390
E-mail: kathy@cornutopia.com
www.corn-utopia.com

A Cornucopia of Corn Snakes

Over the past several decades, corn snakes have risen to claim the title of most commonly kept pet snakes in the world. The many qualities that have contributed to their immense popularity will be examined to demonstrate how they reached that status. The extremes of the current variation in color and patterns will also be shown. A brief history tracing the corn snake's evolution since the late 1950s (when the first 'albino' was found) will be presented as a prime example of a quintessential success story of modern herpetoculture.

Kathy Love has been keeping and breeding *Elaphe guttata* for over a quarter of a century. She is responsible for creating and refining some of the many morphs that have fueled their prevalence in the market. Her continuous work to improve husbandry and breeding techniques, and her renowned efforts of sharing her knowledge with all who have sought her help, have made her a major contributor to corn snakes' dominance of the pet snake trade today.

JOHN MALONE
Department of Biology
University of Texas – Arlington
Box 19498
Arlington, Texas 76019 USA

The Sexual Adventures of the Tropical Treefrogs of the Genus*Smilisca*

Two species of *Smilisca* place eggs in basins while the other four species lay eggs as a surface film in ephemeral pools. I tested two hypotheses concerning the evolutionary function of depositing eggs in basins. The microenvironment modification hypothesis targets potential abiotic benefits of depositing eggs in basins which may lead to increased metamorphic rates. The predator avoidance hypothesis predicts that eggs deposited in basins are segregated from predators and thus have increased survivorship. Results from field observations and egg palatability experiments support a predator avoidance hypothesis but also reveal trade offs from conspecific tadpoles and invertebrate predators that colonize basins.

To understand the origin and distribution of the basin construction reproductive mode, I constructed a phylogeny of *Smilisca* using mtDNA data. These data produced a novel phylogenetic hypothesis which shows that the basin construction reproductive mode is constrained to a monophyletic unit composed of *S. sila* and *S. sordida*.

JAMES C. O'REILLY
Department of Biology
University of Miami
1301 Memorial Drive
202 Cox Science Building
Coral Gables, Florida 33124-0421 USA
Caecilian Natural History

DR. MARK PECKHAM
Bellaire Boulevard Animal Clinic
6213 Bellaire Blvd.
Houston, Texas 77081 USA
Ph:(713) 772-5574

A Day in the Life of a Reptile Veterinarian

Dr. Peckhem will share some of experiences a s a reptile veterinarian. Many topics will be discussed; including how to find a reptile veterinarian, quarantine basics, selected dreaded diseases, and case reports.

JOHN C. PÉREZ and ELDA E. SÁNCHEZ
Natural Toxins Research Center (NTRC)
Texas A&M University-Kingsville
Campus Box 158
Kingsville, TX 78363 USA

A Review of an Internet Database of Venoms Found in the United States

Snake venoms are complex mixtures of pharmacologically important molecules, some of which may have potential therapeutic agents. Snake venoms are rich, stable sources of disintegrins, metalloproteases, and fibrinolytic enzymes. The literature contains many references on how venom components are being used in medicine. Within the United States, there are 27

recognized species of venomous snakes. Despite this rather vast diversity of venomous snakes in North America, most of the biomedical research is conducted on a limited number of the more common venoms (*Crotalus atrox, C. adamanteus, C. viridis viridis, Agkistrodon contortrix contortrix, A. contortrix laticinctus*, and *A. piscivorus piscivorus*). The venoms from many of the North American species are not available for research. Therefore, the composition and potential usefulness in medicine has not been explored. The Natural Toxins Research Center (NTRC) serpentarium presently houses over 400 venomous snakes composed of 25 species and 33 subspecies. These snake venoms are cataloged in an Internet database along with their geographical location, sex, size, biological activities, high performance liquid chromatography profiles and electrophoretic titration profiles. These venoms can be queried through an on-line search routine. The objectives of the NTRC are to: 1) provide reliable information about venoms and related products from a wide variety of venomous snakes, 2) characterize venoms, and 3) develop a database which will be useful to investigators worldwide. Most of the snake venoms in the database are from the North American continent and all the venoms tested thus far are complex mixtures of many different toxins. The database will be expanded to include those antivenoms used to neutralize specific venoms. This information will be useful to the practitioner, herpetologist, poisonous control center technicians and forensic scientists.

ANDREW H. PRICE, Ph.D.
Natural Resources Scientist
Wildlife Diversity Program
Texas Parks and Wildlife Department
4200 Smith School Road
Austin, Texas 78744 USA
Ph:(512) 912-7022 Fax:(512) 912-7058

The Houston Toad: A Case Study in Conservation Biology and Realpolitik

The Houston Toad (*Bufo houstonensis*), a small member of the *Bufo americanus* species group endemic to east-central Texas, has resided longer on state, national, and international endangered species lists than most other taxa. This species disappeared from most of its historically know range following its discovery in the Houston, Texas, area following World War II, and was known from only a single locality during the three decades prior to 1990. Intensive surveys by Texas Parks and Wildlife Department personnel and associates have resulted in the discovery of new populations in 9 counties in central Texas. This species is restricted to deep sandy soils supporting ephemeral wetlands within native post oak/loblolly pine woodlands and savannas that have been subjected to minimal landscape-scale disturbance. Except for 2 sites, nothing is known about the demographics of the newly-discovered populations; the most robust population known still resides in Bastrop County. A total of 2833 adult Houston Toads have been marked with PIT tags in one watershed within Bastrop State Park from 1990-2002. Breeding choruses of up to 200 individuals form under specific climatic conditions over 1-4 nights during February and early March, separated by intervals of several days to several weeks. Individual females are rarely recaptured during the same breeding season, whereas males have been recaptured as often as 22 times in one year. Maximum longevity appears to be 6 years for males and 5 years for females. Individual toads have been recorded traveling distances of up to 1.3 km during the breeding season. Regional climatic regimes have a profound effect upon Houston Toad recruitment and survivorship, compounded by the current fragmented status of the species' populations. The conservation implications of these data and the contentious history of ongoing community efforts to implement a Habitat Conservation Plan for the Houston Toad in Bastrop County under the U.S. Endangered Species Act will be discussed.

MAHMOOD SASA
Instituto Clodomiro Picado
Universidad de Costa Rica
San Jose, Costa Rica.
email:sasa@cro.ots.ac.cr

The Status and Conservation of Crocodilians in Costa Rica: An Upgrade

Two species of crocodilians occur in Costa Rica, sharing wetlands, rivers, and marshes: the American crocodile *Crocodylus acutus*, and the spectacled caiman *Caiman crocodilus*. Both species are sympatric in the country, but microhabitat partition is evident in most localities along their distribution. Despite the long history of herpetological research in Costa Rica, it was not until recent that studies about the captive management, population ecology and behavior have been conducted in the country. In general, both species are common throughout their distribution in the country. Pacific populations of the American crocodile seem to be denser in Costa Rican than in other countries, probably reflecting conservation efforts that began late 1970's. In the Caribbean coast, most studies have focused in the spectacle caiman. Recent interests on the conservation biology and management of crocodilians have produced a national association of professionals, interested in the study of natural populations and management in captivity. In conjunction with colleagues from USA, an extensive campaign to demystify crocodilians has been in place. Nevertheless, recent reports reveal that conservation status of these populations is in jeopardy, especially in the north part of the country. Future research lines and conservation efforts are addressed here.

PATTI SHOEMAKER
Supervisor, Children's Zoo
Houston Zoo
1513 N. MacGregor
Houston, Texas 77030 USA
Ph :(713) 533-6500

The Natural History of the Alligator Snapping Turtle

Long a subject of folklore and southern culture, the Alligator Snapping Turtle, *Macroclemys temminckii*, with its worm-like lure, still fascinates young and young at heart herpetologists alike. Alligator Snapping Turtles are most common in the southeastern United States, from southern Georgia and northern Florida west to Texas and Oklahoma. They can even reach as far north as Illinois, Indiana and Kansas. The habitat of Alligator Snapping Turtles is rivers, lakes, ponds, swamps and bayous of the river systems of the Gulf of Mexico. Not much is known about the behavior and movements of America's largest freshwater turtle, but one thing is clear, the numbers of Alligator Snapping Turtles is declining at an unsustainable rate.

JIM STOUT
Herpetarium Supervisor
Oklahoma City Zoo
2101 NE 50th Street
Oklahoma City, Oklahoma 73111 USA
Ph: (405) 424-3344

Madagascar Canopy Wildlife

In October 2001 the French run "Canopy Raft Program" gave us the opportunity to explore one of the last remaining, primary rain forest in Madagascar. On the Masoala Peninsula we examined the herpetofaunal community with vertical and horizontal transects. We searched in emergent, upper, mid, lower and ground level habitats. Special attention was paid to phytotelmata and the fauna associated within. The Raft Program offered an assortment of unique collecting methods which will also be discussed.

R. KATHRYN VAUGHN, Ph.D. and
JAMES R. DIXON
Curator
Reptiles and Amphibians
Texas Cooperative Wildlife Collection
Texas A&M University
College Station, Texas 77843-2258 USA

Natural history and taxonomic status of Mexican and southwestern United States blind snakes allied with *Leptotyphlops dulcis*

The tiny, burrowing blind snakes of the genus *Leptotyphlops* are represented in the Americas by two species. One of these species, *Leptotyphlops dulcis* is distributed across the southwestern United States and the eastern half of Mexico. Aspects of the natural history of these secretive snakes will be discussed. In addition, the taxonomic status of *Leptotyphlops dulcis dulcis*, *L. dulcis dissectus*, and *L. dulcis myopicus* have been reexamined and updated, and these results will be presented.

HAROLD K. VORIS
Division of Amphibians and Reptiles
Field Museum of Natural History
Chicago, Illinois 60605 USA

Evolution Across Life Zones: Reptiles Invade the Sea

Among vertebrate historians, amphibians are known for colonizing the land while reptiles are renowned for completing the transition to a fully terrestrial way of life. Several key physiological and morphological innovations such as a leathery egg, and water-conserving kidneys and integument, made the latter transition possible. Since achieving this great evolutionary milestone many reptile lineages have experimented with a return to the sea. Why has this happened? A comparison of the various evolutionary experiments and an exploration of the common trends reveal that exploitation of a new food source seems to be a common factor and perhaps a driving force in the reptiles' return to an aquatic environment. This comparison also reveals that the return to the sea has been accompanied--not by reversals to previous conditions--but by novel adaptations that accomplish the old functions, as exemplified by the variety of specializations among the sea snakes. If abundant nutritional resources have been an important draw, what has limited these evolutionary reversals? A working hypothesis involving predation is explored to begin to try to answer this question.

Edward J. Wozniak D.V.M., Ph.D.
Venom-derived Disintegrins: A New Generation of Anti-tumor Peptides with Potential Therapeutic Value

Disintegrins are a class of non-toxic venom-derived peptides that are potent inhibitors of platelet aggregation. The mechanism of platelet aggregation inhibition is brought about by a complex cascade of subcellular reactions that are initiated by disintegrin engagement of integrin receptors on the platelet surfaces. Through their interaction with the integrins on tumor cells, disintegrins have recently been shown to exhibit anti-cancer activities. Some disintegrins have been shown to arrest tumor growth and prevent metastasis by inhibiting cell proliferation, blocking cell motility, arresting vasculogenesis, and inducing endothelial cell apoptosis in human neoplasms. The net effect of these changes is a smaller, more localized tumor that is less likely to metastasize. Because of their therapeutic potential in the treatment of some cancers, there is biotechnological interest in developing some disintegrins as anti-neoplastic drugs.

While numerous disintegrins have been isolated from Asian Crotalinae, with the exception of the southern copperhead from which the anti-tumor peptide contortrostatin was derived, few disintegrin surveys have been done on New World species and no comparative studies have been done at the specific, subspecific and population levels of resolution (biotypes). With the rather extensive degree of geographic isolation and evolutionary divergence evident in the New World Crotalinae, the probability for unique disintegrins with anti-tumor properties in the vast array of known genera and species is predicted to be high. The purpose of this study was to characterize and compare the primary structure of disintegrins in a specified group of New World Crotalinae at the specific, subspecific and population levels of resolution. The specific aims were to test the following three hypotheses: 1) The structural features of venom-derived disintegrins vary between species and subspecies of the New World *Agkistrodon* species complex, *Crotalus lepidus and Atropoides nummifer nummifer*, 2) Structurally different disintegrins will have different integrin affinities and biological activities, 3) Some members of the New World Crotalinae undergo ontogenetic shifts in disintegrin expression.

Venom samples from each age-specific snake biotype were collected in chilled receptacles, centrifuged, sterile filtered and snap frozen at -70 C. Aliquots of raw venom were analyzed by polyacrylamide gel electrophoresis and the disintegrin fractions identified by Western blot using rabbit anti-contortrostatin antibodies. Fractions with proteolytic activity were identified by zymography. Initial analysis of the structural features of each disintegrin was accomplished by characterizing the corresponding mRNA sequences by high fidelity RT-PCR using custom designed oligonucleotide primers. Each resulting cDNA construct was inserted into a plasmid and cloned in competent JM-109 *E. coli* from which it will be recovered for sequence analysis. The amino acid sequences deduced from the cloned cDNA sequence information will be used in the initial comparison of primary structures.

Western blot analysis demonstrated similarly sized disintegrin-positive proteins (45-55 kDa and 13-16 kDa) in the venoms from all of the copperhead and cottonmouth subspecies in the study population. Both *A. bilineatus bilineatus* and *A. taylori* had disintegrin fractions that were easily distinguishable (150 kDa and 14.5-15 kDa) from those in the copperheads and the western cottonmouth. All of the non-enzymatic disintegrin-positive fractions identified in *Agkistrodon* sp. venoms approximate dimeric variants in size. *Crotalus lepidus lepidus* venom was found to contain disintegrin-positive proteins approximately 55 kDa and 8 kDa. The 8 kDa band is approximately the size of a monomeric disintegrin. Zymographic analysis with casein and type-A gelatin demonstrated proteolytic activity in all of the high molecular weight disintegrin-positive fractions in each of the venoms sampled. Interestingly, no disintegrins that were reactive with anti-contortrostatin antibody were demonstrated in *A. nummifer nummifer* venom. This finding suggests that this family of proteins to be either structurally different from those in other North American and Mexican Crotalinae of lacking all together.

High fidelity RT-PCR demonstrated similar RNA sequences in the venom glands of all of the coralline species sampled including *A. nummifer nummifer*. All resulting cDNA sequences were approximately 175 bp in size which very closely approximates the predicted size of the product sequence in contortrostatin. Each resulting cDNA construct (N=10 thus far) was successfully incorporated into a plasmid and used to transform competent *E. coli*. The average transformation efficiency was with the protocol used in our laboratory was 96%. This is an ongoing study.

Of Golden Toads & Serpents' Roads

Paul Freed

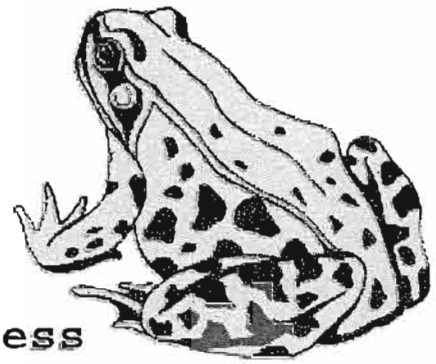
This volume invites readers to join a seasoned researcher as he "goes herping." After nearly twenty years of traveling around the globe searching for toads, frogs, salamanders, snakes, lizards, and turtles, herpetologist Paul Freed pauses to tell stories of his adventures finding and collecting reptiles and amphibians from the tropics of Costa Rica to the deserts of Namibia.

Whether confronting scorpions, beefworms, army ants, or venomous snakes, Freed conveys a contagious enthusiasm for the outdoors and all that lives in it. With humor and gratitude, he embraces both the hardships and the rewards of being in the field. His tales of discovery are also travel stories, and by sharing the experiences of his trips across the globe—"the successful ones and those not so successful"—Freed encourages the adventurer in all of us.

As he ponders on a writhing knot of golden toads—a species that may now be extinct—or the twitching blue tail broken from a girdled lizard, Freed introduces conservation issues particular to specific locales and those shared across the global village. Through his experiences, readers learn a variety of exotic wildlife collection techniques, some of which represent ingenious improvising under hazardous conditions and some of which are downright humorous. Readers share the excitement of scientific discoveries, the thrill of international travel to remote and fascinating regions, and the opportunity to encounter both rare and abundant wildlife in its natural habitats.

Armchair naturalists, amateur herpers, and people interested in exotic travel, ecotourism, and scientific adventure will find this book just as appealing and informative as will professional and academic herpetologists.

"... combines the spirit of adventure with natural history and science. Readers are lured by exotic travel and his storytelling skills and ultimately come away with a better understanding of wildlife, research, and the plight of amphibian and reptile conservation in a compelling and interesting manner."
—*Russell A. Mittermeier, President, Conservation International*

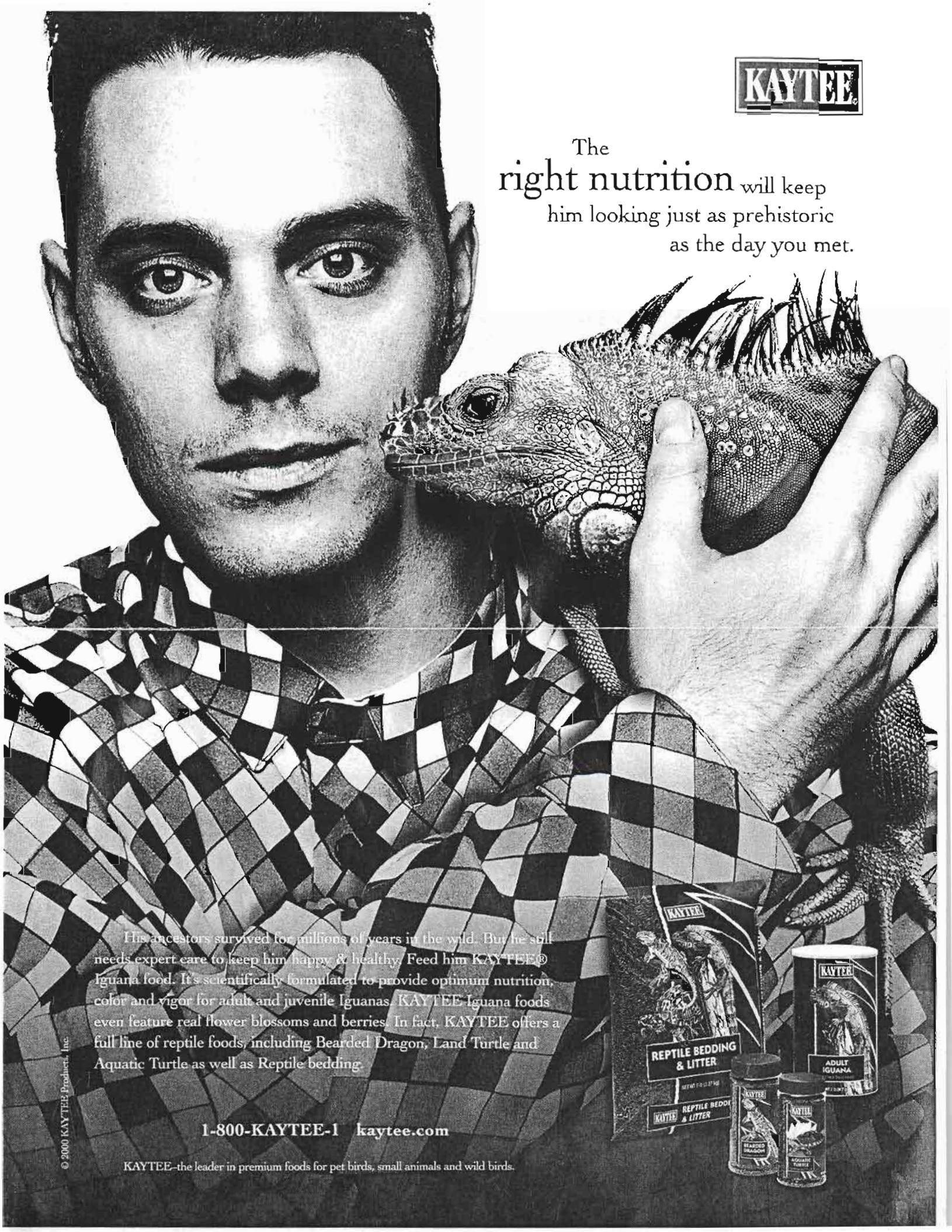


Number Thirty-four:
Louise Lindsey Merrick Natural Environment Series

55 color photos
1-58544-248-8 cloth, $32.95
1-58544-271-2 paper, $18.95

Texas A&M University Press

College Station, Texas 77843-4354 800-826-8911 Fax: 888-617-2421 www.tamu.edu/upress


KAYTEE
The
right nutrition will keep
him looking just as prehistoric
as the day you met.
His ancestors survived for millions of years in the wild. But he still needs expert care to keep him happy & healthy. Feed him KAYTEE® Iguana food. It's scientifically formulated to provide optimum nutrition, color and vigor for adult and juvenile Iguanas. KAYTEE Iguana foods even feature real flower blossoms and berries. In fact, KAYTEE offers a full line of reptile foods, including Bearded Dragon, Land Turtle and Aquatic Turtle as well as Reptile bedding.
REPTILE BEDDING & LITTER
ADULT IGUANA
1-800-KAYTEE-1 kaytee.com
KAYTEE–the leader in premium foods for pet birds, small animals and wild birds.
© 2000 KAYTEE Products, Inc.