# “Current and Emerging Infectious Diseases of Reptiles: Comparative Medicine at the Crossroads of Vertebrate Evolution”


**Elliott Jacobson, DVM, PhD, DACZM**

**College of Veterinary Medicine**

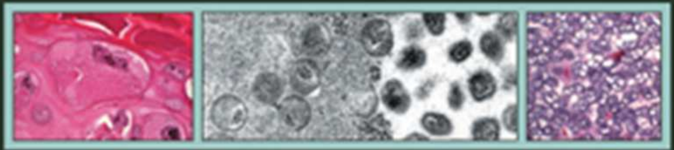
**University of Florida**


Veterinary Medicine, Biological Sciences

## INFECTIOUS DISEASES AND PATHOLOGY OF REPTILES

Color Atlas and Text

ELLIOT R. JACOBSON



*Infectious Diseases and Pathology of Reptiles: Color Atlas and Text* compiles the latest information on every aspect of the pathology and diagnosis of infectious diseases in reptiles. It offers the most thorough review of the biology, anatomy, and histology of reptiles and covers viral, bacterial, fungal, and parasitic diseases as well as methods for isolating pathogens. It features an inclusive collection of approximately 1400 images, many of which come directly from the editor's esteemed collection documenting more than 30 years' experience in the research of infectious diseases and veterinary care of reptiles.

With up-to-the-minute data, never-before-seen images, and a stellar panel of contributors, *Infectious Diseases and Pathology of Reptiles* is the definitive resource for veterinarians, biologists, and researchers involved in the study of pathogens infecting reptiles.

- Features high-quality, full-color photos depicting normal and pathological anatomy and physiology
- Presents unique gross, light, and electron microscopic images of pathogens and diseases
- Offers the most complete single source for color images of reptile histology
- Provides an overview of the use of electron microscopy in diagnosis including numerous electron micrographs of lesions
- Introduces the necessity of molecular methods for diagnosis
- Addresses the mechanism of reptile immunology
- Outlines serodiagnostics and the use of immunological reagents specifically designed for reptiles including indirect enzyme-linked immunosorbent assays (ELISA)

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INFECTIOUS DISEASES AND PATHOLOGY OF REPTILES

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# INFECTIOUS DISEASES AND PATHOLOGY OF REPTILES

## Color Atlas and Text

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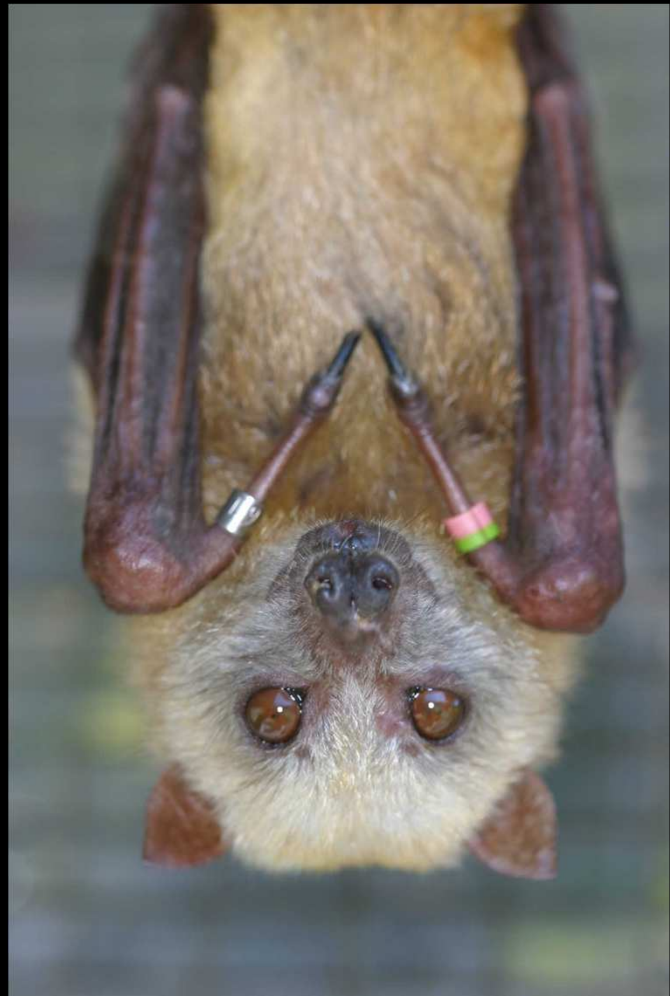

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# BIOLOGY OF REPTILES

## Animal Kingdom

Present day reptiles comprise a small portion of the Animal Kingdom, having approximately 6,500 species. This compares with approximately 20,000 fish 10,000 birds, and 7,000 species of mammals. Present day reptiles are divided into the 4 orders: Chelonia, Crocodilia, Rhynchocephalia, and Squamata.

## Invertebrates

1 million+ species

## Vertebrates

a. Agnatha - 45 species
b. Chondrichthyes - 675 species
c. Osteichthyes - 21,000 spp
d. Amphibia – 4,500 species
e. Reptilia - 7,500 species
f. Aves - 10,000 species
g. Mammalia - 7,000 species

# CLASSIFICATION OF REPTILIA

Present day reptiles are grouped into 4 orders. During the Mesozoic there were 17 orders. The oldest present day order is Chelonia. Fossil chelonians can be traced back over 100 million years. The most recent group is Squamata, the lizards and snakes. The majority of present day reptiles are within this group.

**Chelonia** - turtles and tortoises

250 species

**Crocodylia** - alligators, caiman, crocodiles, caiman, gharial

23 species

**Rhynchocephalia** - tuatara - 2 species

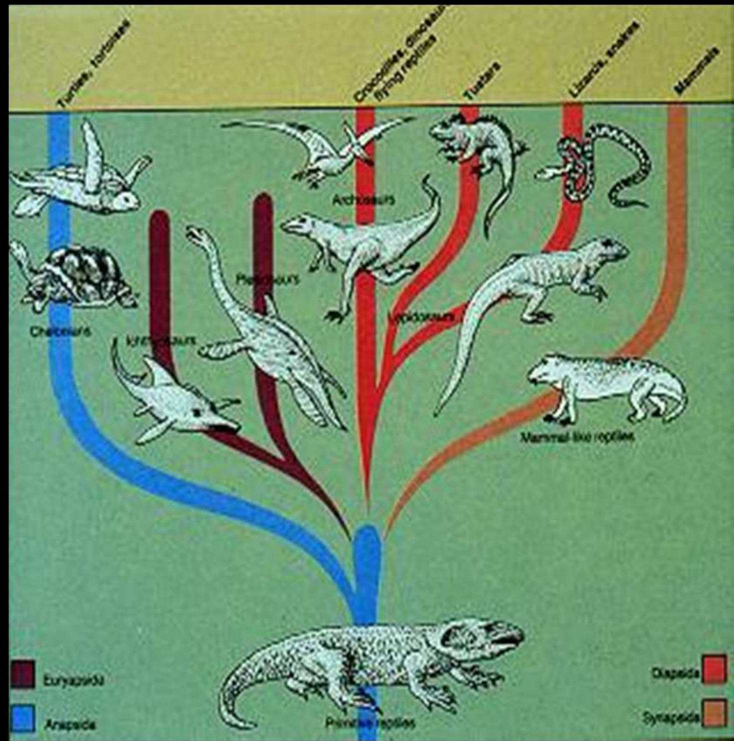
**Squamata**

- Lacertilia - lizards - 4,200 species
- Ophidia - snakes - 3,100 species
- Amphisbaenia - 140 species

# BIOLOGY OF REPTILES

## Reptile Phylogeny

**During the Mesozoic there were 17 orders of reptiles. While most were terrestrial, there were several aquatic and 1 order of flying reptiles. Of these orders, there are 4 extant groups. At the right is a phylogenetic representation of the orders of reptiles. All come off different lines and are distantly related. Crocodilians come off a line that gave rise to the dinosaurs. Crocodilians are more closely related to birds than to other groups of reptiles.**




**From: Halliday, T., and Adler, K. 1987. The Encyclopedia of Reptiles and Amphibians. Facts on File Inc., N.Y. Pg. 62.**

# Phylogenomics of nonavian reptiles and the structure of the ancestral amniote genome

**Andrew M. Shedlock[†‡], Christopher W. Botka[§], Shaying Zhao[¶‖], Jyoti Shetty[¶††], Tingting Zhang[‡‡], Jun S. Liu[‡‡], Patrick J. Deschavanne[§§], and Scott V. Edwards[†]**

[†]Department of Organismic and Evolutionary Biology, Museum of Comparative Zoology, Harvard University, 26 Oxford Street, Cambridge, MA 02138; [§]Research Division, Joslin Diabetes Center, Harvard Medical School, One Joslin Place, Boston, MA 02215; [¶]The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850; [‡‡]Department of Statistics, Harvard University, 1 Oxford Street, Cambridge, MA 02138; and [§§]Equipe de Bioinformatique Genomique et Moleculaire, Institut National de la Santé et de la Recherche Médicale (INSERM), 2 Place Jussieu, 75005 Paris, France

Edited by David B. Wake, University of California, Berkeley, CA, and approved December 26, 2006 (received for review July 24, 2006)

**We report results of a megabase-scale phylogenomic analysis of the Reptilia, the sister group of mammals. Large-scale end-sequence scanning of genomic clones of a turtle, alligator, and lizard reveals diverse, mammal-like landscapes of retroelements and simple sequence repeats (SSRs) not found in the chicken. Several global genomic traits, including distinctive phylogenetic lineages of CR1-like long interspersed elements (LINEs) and a paucity of A-T rich SSRs, characterize turtles and archosaur genomes, whereas higher frequencies of tandem repeats and a lower global GC content reveal mammal-like features in *Anolis*. Nonavian reptile genomes also possess a high frequency of diverse and novel 50-bp unit tandem duplications not found in chicken or mammals. The frequency distributions of ≈65,000 8-mer oligonucleotides suggest that rates of DNA-word frequency change are an order of magnitude slower in reptiles than in mammals. These results suggest a diverse array of interspersed and SSRs in the common ancestor of amniotes and a genomic conservatism and gradual loss of retroelements in reptiles that culminated in the minimalist chicken genome.**

Little is known about the large-scale structure of nonavian reptile genomes at the sequence level. Alligator and turtle genome sizes are ≈30% smaller than human, ≈50% larger than chicken, and only ≈12% larger than *Anolis*, whose genome size is close to the mean for nonavian reptiles. Unlike alligator genomes, the anole, painted turtle, and chicken contain a significant number of microchromosomes (7), which we expect would be gene rich as reported for chickens (8) and the soft-shelled turtle (9). In general, it is unknown how the macrochromosomes of reptiles differ from those of mammals (10) and those of the nonavian reptiles investigated here. The turtle and alligator species investigated here have environmental as opposed to genetic sex determination, and sex determination in *Anolis* is inferred to be genetic based on some karyological evidence (11). Several retroelement lineages have been characterized in turtles and other reptiles (12–15). Projects in progress will produce genome sequences for another bird, the Zebra Finch, *Taeniopygia guttata*, and a lizard, *Anolis carolinensis*. In the meantime, our goal in this project was to quickly amass a moderate database of primary sequence distributed throughout the genomes of genomically understudied lineages, which can reveal numerous

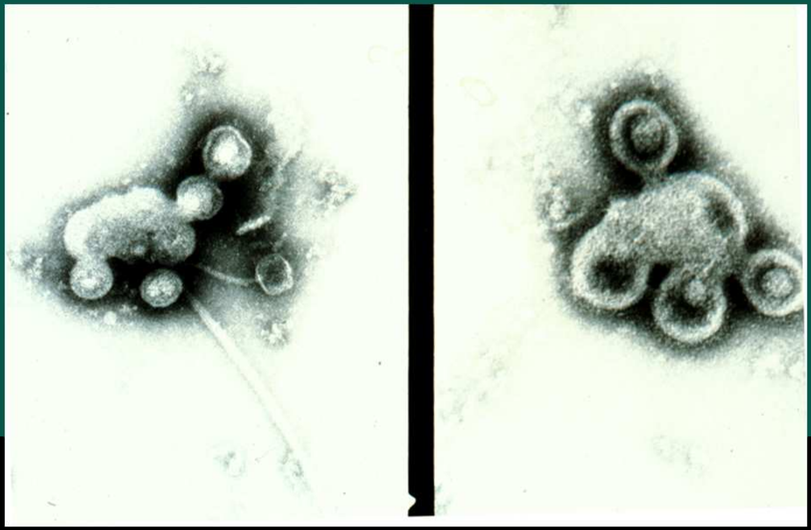
# Families of Viruses Known to Infect Reptiles

- Herpesviridae
- Adenoviridae
- Poxviridae
- Papovaviridae
- Circoviridae
- Paravoviridae
- Iridoviridae
- Flaviviridae

- Picornaviridae
- Caliciviridae
- Togaviridae
- Parymyxoviridae
- Rhabdoviridae
- Reoviridae
- Retroviridae

# VIRAL INFECTIONS OF REPTILES

- Routine Histopathology
- Immunohistochemical staining
- Transmission Electron Microscopy
- Negative Staining Electron Microscopy
- Cell culture
- Enzyme Assays
- Serology
- PCR

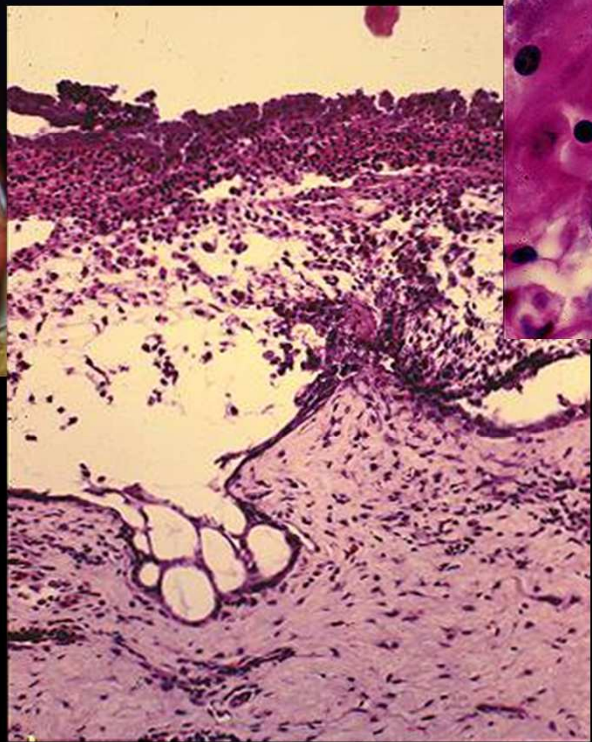


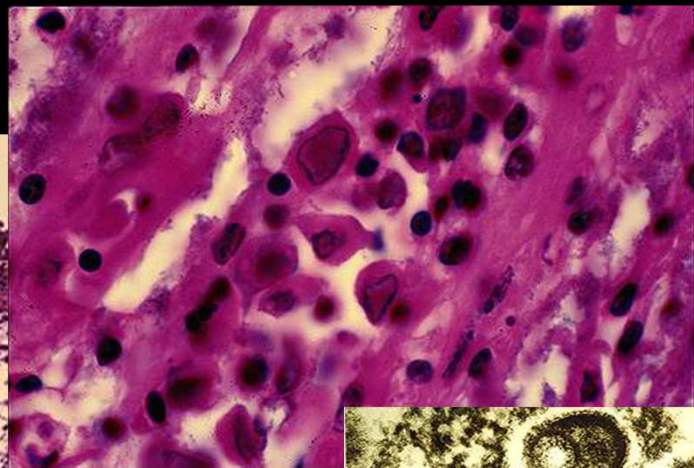
# HERPESVIRUS INFECTIONS OF REPTILES

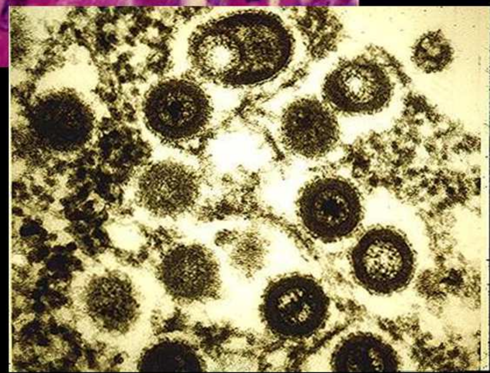
- Hepatic necrosis herpesviruses of fresh water turtles
- Snake venom gland herpesvirus
- Herpesvirus stomatitis in tortoises
- Marine turtle herpesviruses

# VIRAL INFECTIONS OF REPTILES

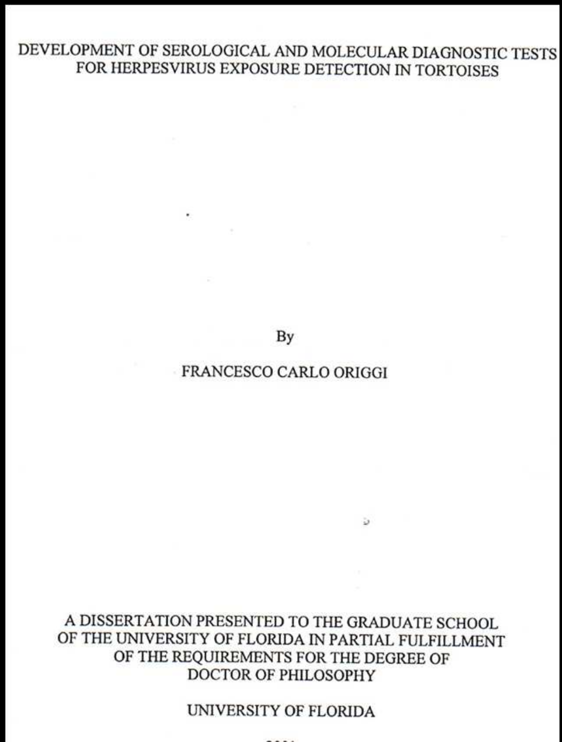
**HERPESVIRUS STOMATITIS/**
**PHARYNGITIS IN TORTOISES**







# HERPESVIRUS STOMATITIS/PHARYNGITIS IN TORTOISES

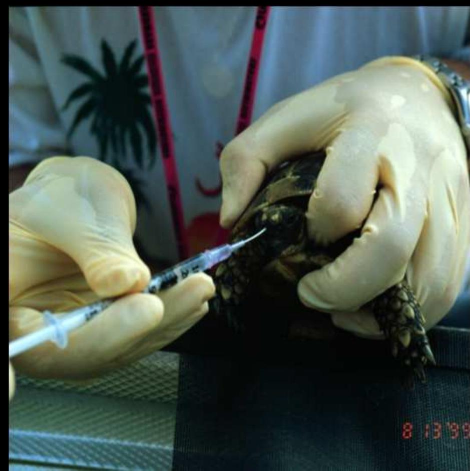

DEVELOPMENT OF SEROLOGICAL AND MOLECULAR DIAGNOSTIC TESTS FOR HERPESVIRUS EXPOSURE DETECTION IN TORTOISES

By

FRANCESCO CARLO ORIGGI

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA



# Francesco Origii
# Herpesvirus PhD Research Project

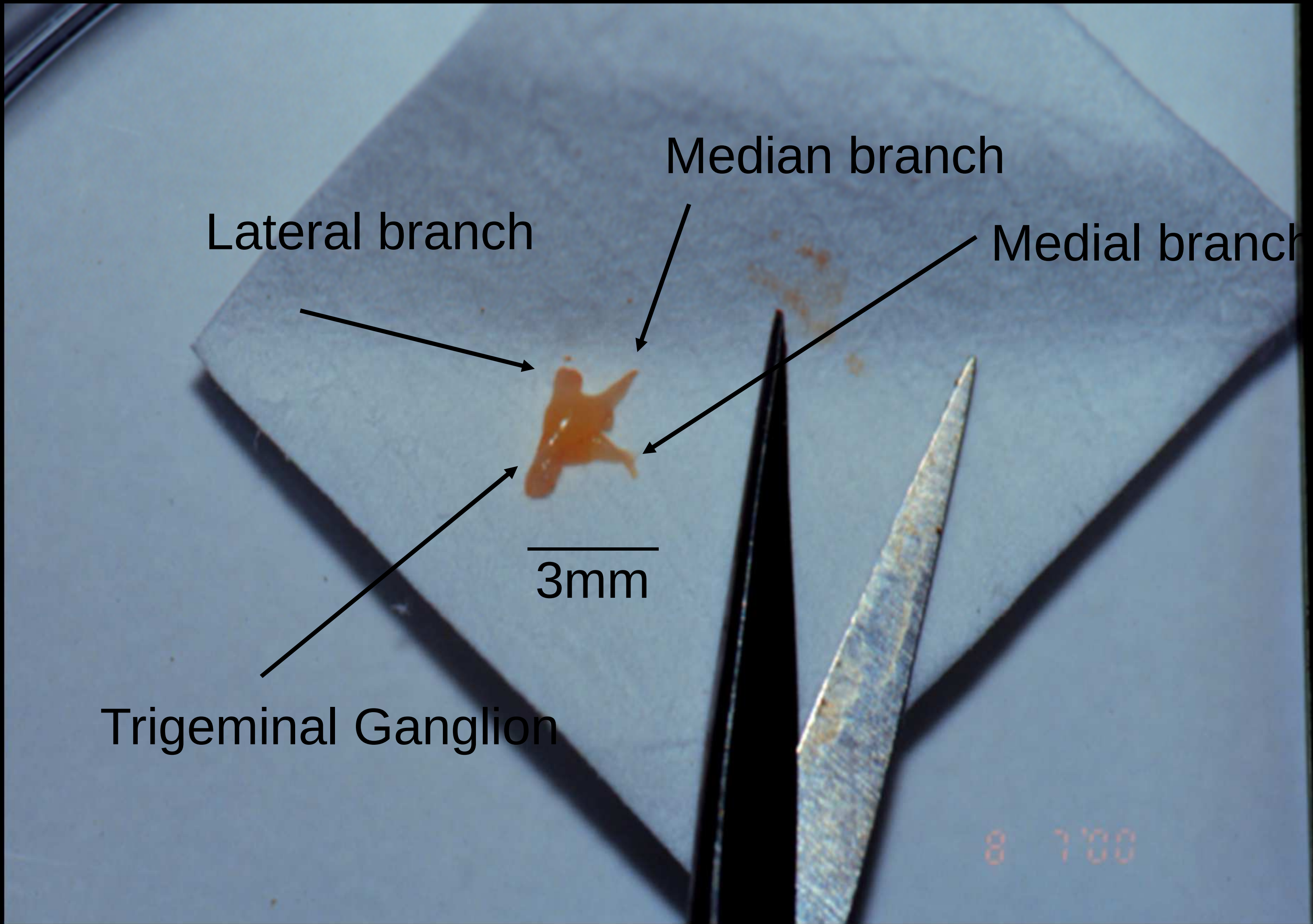
- Immunoperoxidase
- <u>ELISA</u>
- Tortoise herpesvirus transmission study
- Virus Cloning
- Viral genome sequencing
- PCR and RT-PCR
- Latency investigation
- Recombinant antigens

## Conjunctivitis

## Oral Discharge

## Oral Plaques

# Virus tropism investigation



# ELISA For Detecting Herpesvirus Exposure

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2001, p. 3156–3163 Vol. 39, No. 9
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.9.3156–3163.2001


## Enzyme-Linked Immunosorbent Assay for Detecting Herpesvirus Exposure in Mediterranean Tortoises (Spur-Thighed Tortoise [*Testudo graeca*] and Hermann's Tortoise [*Testudo hermanni*])†

F. C. ORIGGI,[1,2]* P. A. KLEIN,[3] K. MATHES,[4] S. BLAHAK,[5] R. E. MARSCHANG,[6] S. J. TUCKER,[1] AND E. R. JACOBSON[1]

*Department of Small Animal Clinical Sciences,[1] Department of Pathobiology,[2] and Department of Pathology,[3] University of Florida, Gainesville, Florida 32610-0126, and Klinik fuer kleine Haustiere Tieraerztliche Hochschule, Hannover,[4] Staatliches Veterinaruntersuchungsamt, Detmold,[5] and Institute for Avian and Reptile Medicine, Justus Liebig University, Giessen,[6] Germany*

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**An enzyme-linked immunosorbent assay (ELISA) was developed for the detection of antibodies to a herpesvirus associated with an upper respiratory tract disease in Mediterranean tortoises [spur-thighed tortoise (*Testudo graeca*) and Hermann's tortoise (*Testudo hermanni*)]. This serodiagnostic test was validated through a hyperimmunization study. The mean of the $A_{405}$ readings of the plasma samples collected at time zero of the hyperimmunization study plus three times the standard deviation was used as the cutoff for seropositivity in tortoises. ELISA results were compared to serum neutralization (SN) values for the same samples by using the McNemar test. The results obtained by SN and ELISA were not significantly different ($P > 0.05$). This new ELISA could be used as an important diagnostic tool for screening wild populations and private and zoo collections of Mediterranean tortoises.**

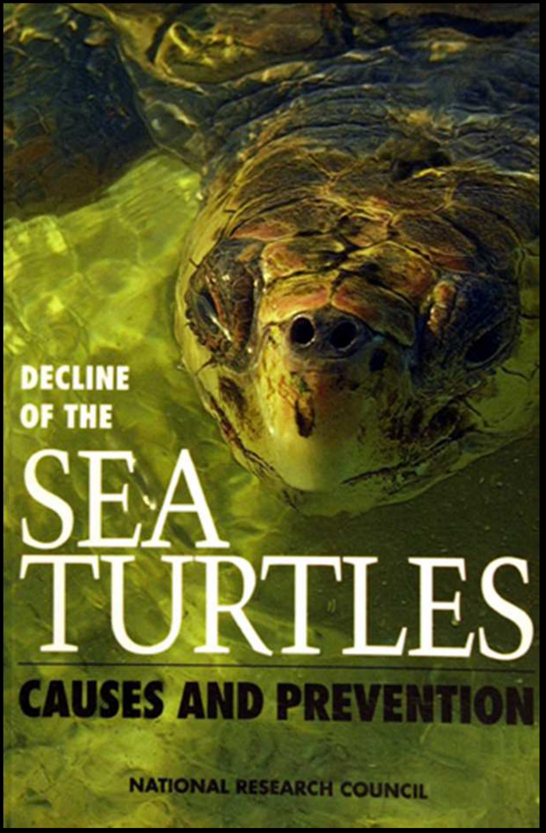
# PCR Summary Results

| | |
|---|---|
| Total positive samples CNS: | 20 |
| Total Positive samples Resp + Dig. Tr. | 13 |
| Total Positive samples Urogen. Tract. | 5 |
| Total Positive samples misc. organs | 2 |
| Total Positive samples | 40 |
| Total Samples Collected | 126 |

# THREATS TO SEA TURTLE CONSERVATION

## Environmental Problems

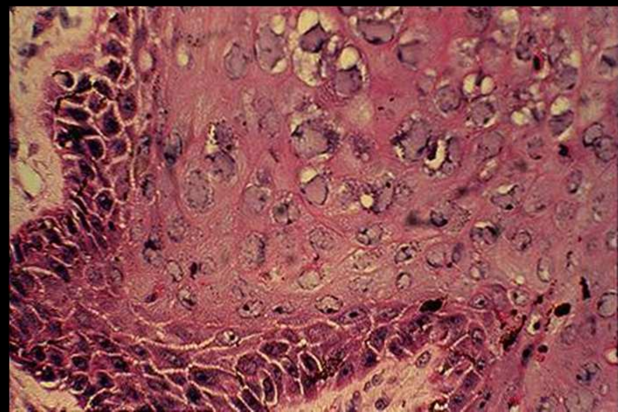
- Habitat Loss
- Fish hooks
- Boat Impact Injuries
- Ingestion of plastics and tar
- Shark Injuries



# Marine Turtle Herpesviruses

**Green Turtle**

**Gray Patch Disease**

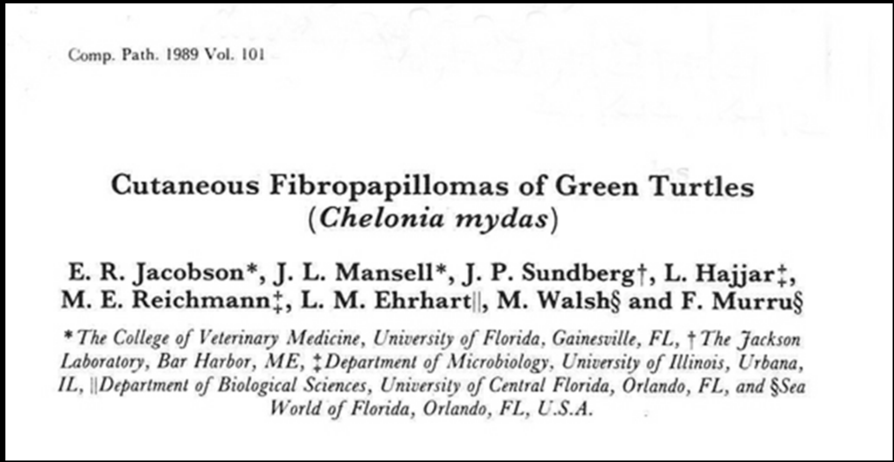


# Green Turtle – *Chelonia mydas*

# Lung, Eye, and Trachea (LET) Disease

**Jacobson, E.R., Gaskin, J.M., Roelke, M., Greiner, E.C., Allen, J. 1986. Conjunctivitis, Tracheitis, and Pneumonia Associated with Herpesvirus Infection in Green Sea Turtles. J. Amer. Vet. Med. Assoc. 189:1020-1023.**

# Marine Turtle Herpesviruses

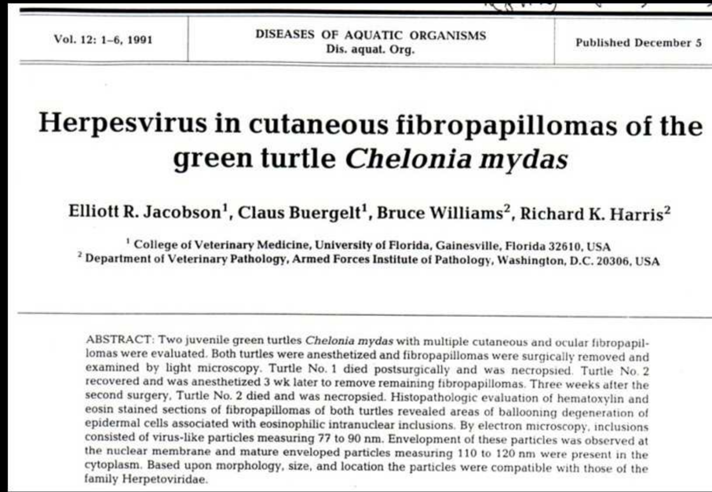


Comp. Path. 1989 Vol. 101

## Cutaneous Fibropapillomas of Green Turtles (*Chelonia mydas*)

E. R. Jacobson*, J. L. Mansell*, J. P. Sundberg†, L. Hajjar‡, M. E. Reichmann‡, L. M. Ehrhart||, M. Walsh§ and F. Murru§

**The College of Veterinary Medicine, University of Florida, Gainesville, FL, †The Jackson Laboratory, Bar Harbor, ME, ‡Department of Microbiology, University of Illinois, Urbana, IL, ||Department of Biological Sciences, University of Central Florida, Orlando, FL, and §Sea World of Florida, Orlando, FL, U.S.A.*



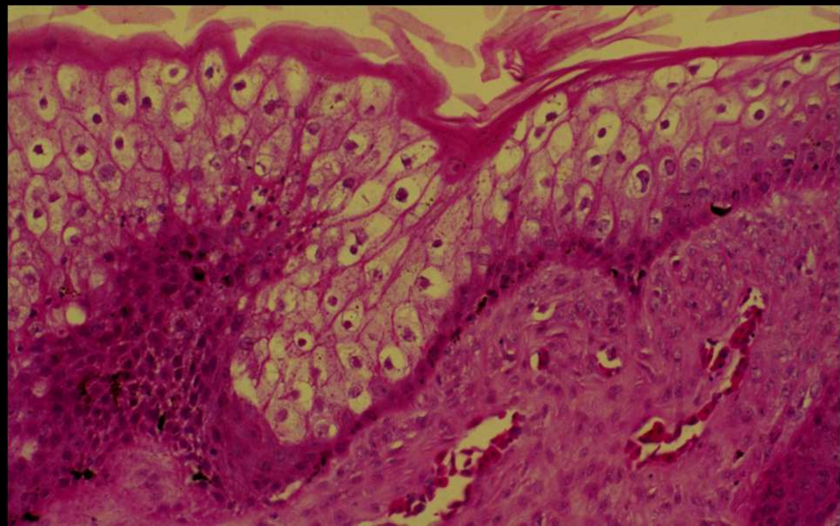
Vol. 12: 1–6, 1991 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published December 5

## Herpesvirus in cutaneous fibropapillomas of the green turtle *Chelonia mydas*

Elliott R. Jacobson[1], Claus Buergelt[1], Bruce Williams[2], Richard K. Harris[2]

[1] College of Veterinary Medicine, University of Florida, Gainesville, Florida 32610, USA
[2] Department of Veterinary Pathology, Armed Forces Institute of Pathology, Washington, D.C. 20306, USA

ABSTRACT: Two juvenile green turtles *Chelonia mydas* with multiple cutaneous and ocular fibropapillomas were evaluated. Both turtles were anesthetized and fibropapillomas were surgically removed and examined by light microscopy. Turtle No. 1 died postsurgically and was necropsied. Turtle No. 2 recovered and was anesthetized 3 wk later to remove remaining fibropapillomas. Three weeks after the second surgery, Turtle No. 2 died and was necropsied. Histopathologic evaluation of hematoxylin and eosin stained sections of fibropapillomas of both turtles revealed areas of ballooning degeneration of epidermal cells associated with eosinophilic intranuclear inclusions. By electron microscopy, inclusions consisted of virus-like particles measuring 77 to 90 nm. Envelopment of these particles was observed at the nuclear membrane and mature enveloped particles measuring 110 to 120 nm were present in the cytoplasm. Based upon morphology, size, and location the particles were compatible with those of the family Herpetoviridae.



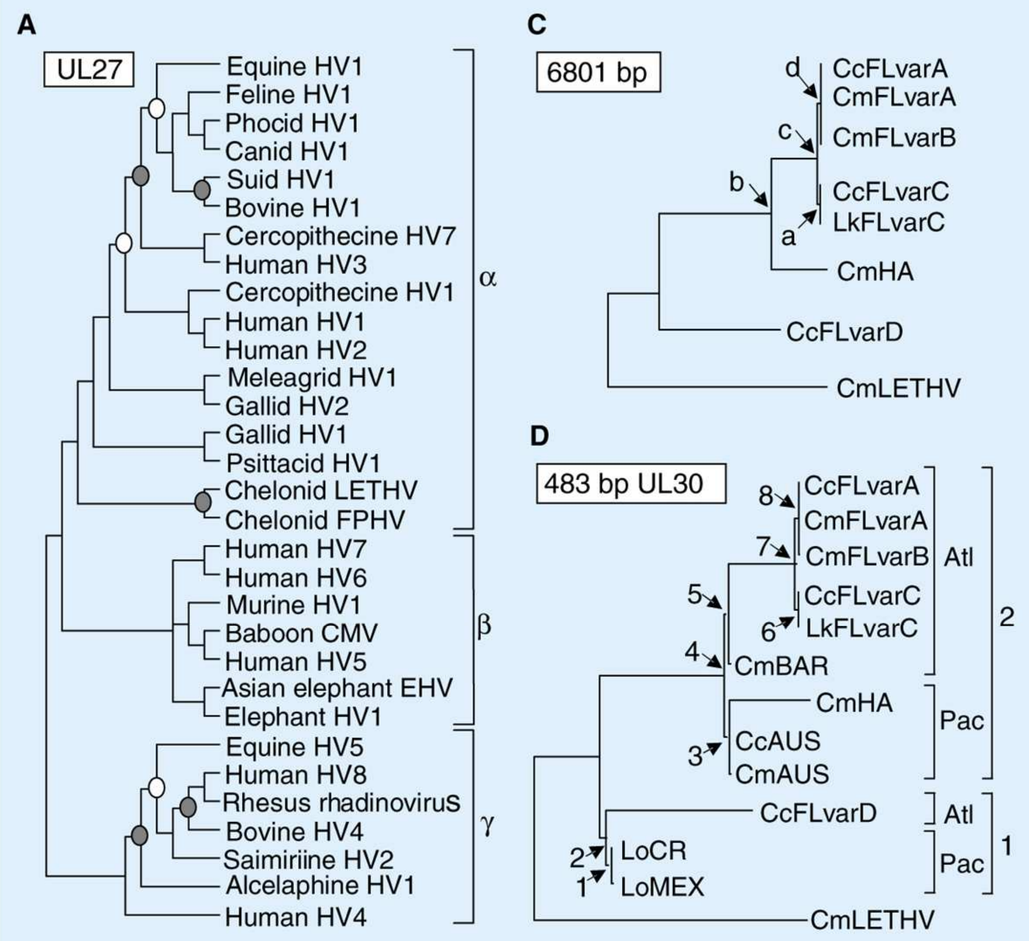
Correspondence

## Tumor outbreaks in marine turtles are not due to recent herpesvirus mutations

Larry Herbst[1*], Ada Ene[1,2], Mei Su[2], Rob Desalle[3] and Jack Lenz[2*]

Marine turtle fibropapillomatosis is a devastating, transmissible disease characterized by multiple cutaneous and visceral fibrovascular tumors [1]. It has emerged with high prevalence since the 1980s and threatens the survival of several species of marine turtles. A herpesvirus, here called chelonid fibropapilloma-associated herpesvirus (C-FP-HV), is present in all naturally occurring tumors but not in unaffected turtles. It is also present in all tumors experimentally induced with cell-free tumor filtrates [2–5].

We assembled 43,843 bp of sequence of the C-FP-HV genome (GenBank AY644454), which encompasses 20 genes that are orthologous to cognate genes of other alpha-herpesviruses and organized in a similar fashion. In addition, the sequence included a novel 4 kb segment between UL15B and UL18 for which there is no equivalent in other herpesviruses (Supplemental Data). Phylogenetic analyses based on UL27, which has been widely used for herpesvirus phylogeny [6], show that C-FP-HV is related to other alpha-herpesviruses. It is most closely related to a non-oncogenic, marine turtle herpesvirus, C-LET-HV (Figure 1A). This UL27-based phylogeny agrees with that based on parts of UL29 and UL30 [3,7,8]. Alpha-herpesviruses are known to show extensive coevolution with their hosts [9–11]. The turtle virus lineage appears to have diverged before the separation of avian and mammalian alpha-herpesviruses (Figure 1A), suggesting that these herpesviruses have been unique




**B**

| Variant | Location-host (N) | Differences in 6801 bp |
|---|---|---|
| FL-A | FL-Cm (6), FL-Cc (6), NC-Cc (1) | Reference sequence |
| FL-B | FL-Cm (3) | 1 bp, 0.02% |
| FL-C | FL-Cc (1), FL-Lk (1) | 9 bp, 0.13% |
| FL-D | FL-Cc (1), NC-Cc (1) | 383 bp + one 3 bp ins, 5.6% |
| HA | HA-Cm (5) | 145 bp + one 3 bp ins, 2.2% |

Current Biology

Available online at www.sciencedirect.com
ScienceDirect
Veterinary Microbiology 126 (2008) 63–73
veterinary microbiology
www.elsevier.com/locate/vetmic

# Two herpesviruses associated with disease in wild Atlantic loggerhead sea turtles (*Caretta caretta*)

Brian A. Stacy[a,*], James F.X. Wellehan[a], Allen M. Foley[b], Sadie S. Coberley[c], Lawrence H. Herbst[d], Charles A. Manire[e], Michael M. Garner[f], Milagros D. Brookins[g], April L. Childress[a], Elliott R. Jacobson[a]

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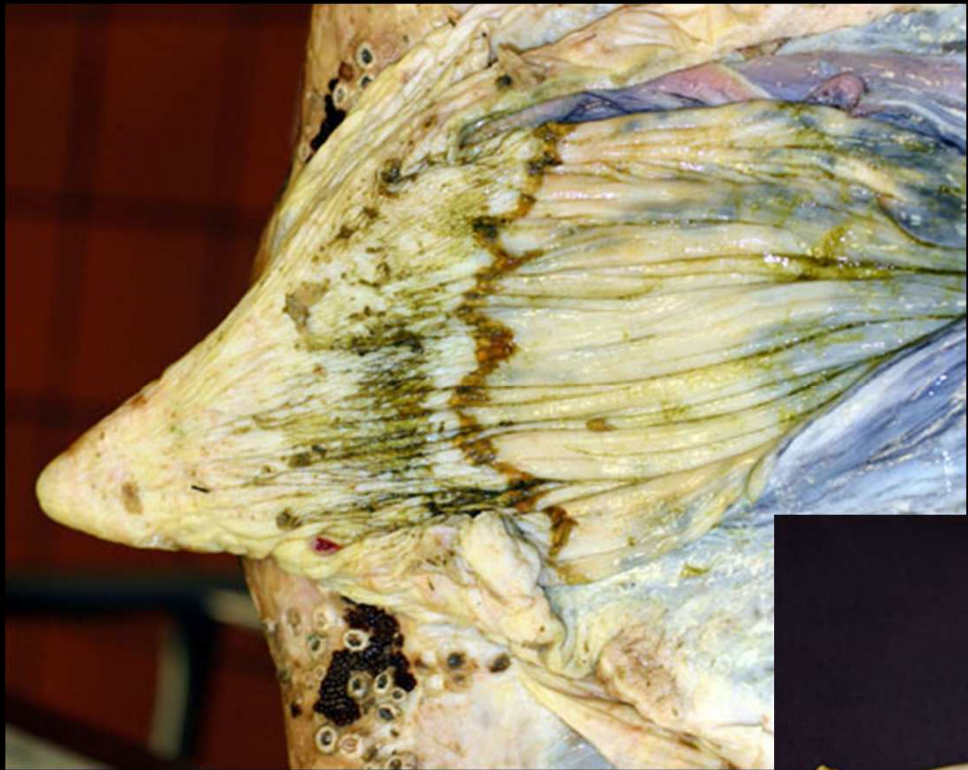
Received 23 April 2007; received in revised form 24 June 2007; accepted 3 July 2007

**Abstract**

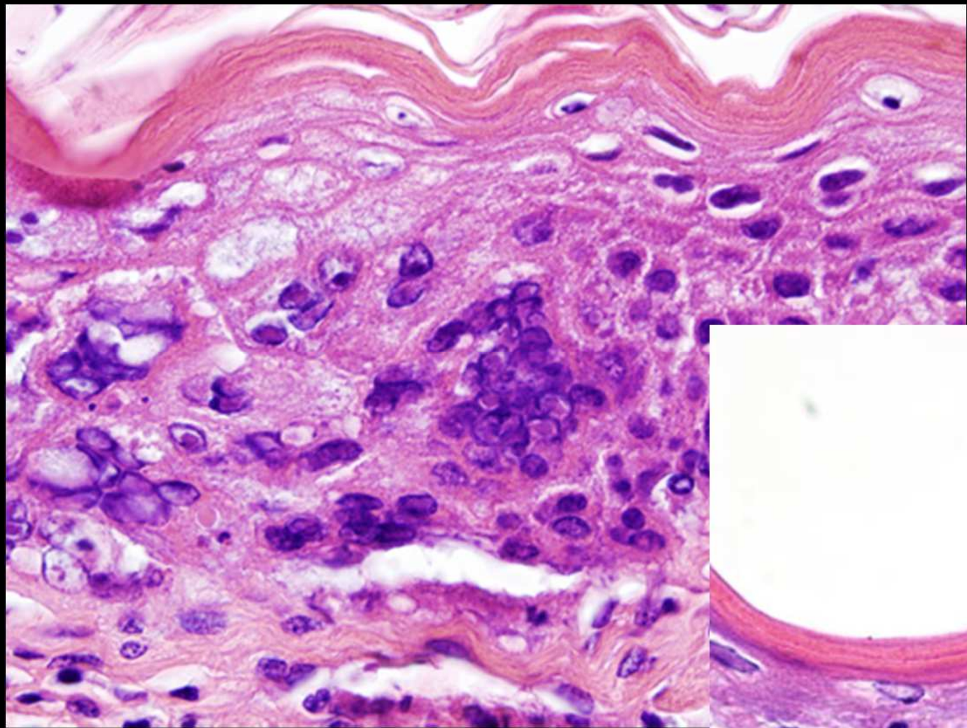
Herpesviruses are associated with lung–eye–trachea disease and gray patch disease in maricultured green turtles (*Chelonia mydas*) and with fibropapillomatosis in wild sea turtles of several species. With the exception fibropapillomatosis, no other diseases of wild sea turtles of any species have been associated with herpesviral infection. In the present study, six necropsied Atlantic loggerhead sea turtles (*Caretta caretta*) had gross and histological evidence of viral infection, including oral, respiratory, cutaneous, and genital lesions characterized by necrosis, ulceration, syncytial cell formation, and intranuclear inclusion bodies. Nested polymerase chain reaction targeting a conserved region of the herpesvirus DNA-dependent-DNA polymerase gene yielded two unique herpesviral sequences referred to as loggerhead genital-respiratory herpesvirus and loggerhead orocutaneous herpesvirus. Phylogenetic analyses indicate that these viruses are related to and are monophyletic with other chelonian herpesviruses within the subfamily *α-herpesvirinae*. We propose the genus Chelonivirus for this monophyletic group of chelonian herpesviruses.

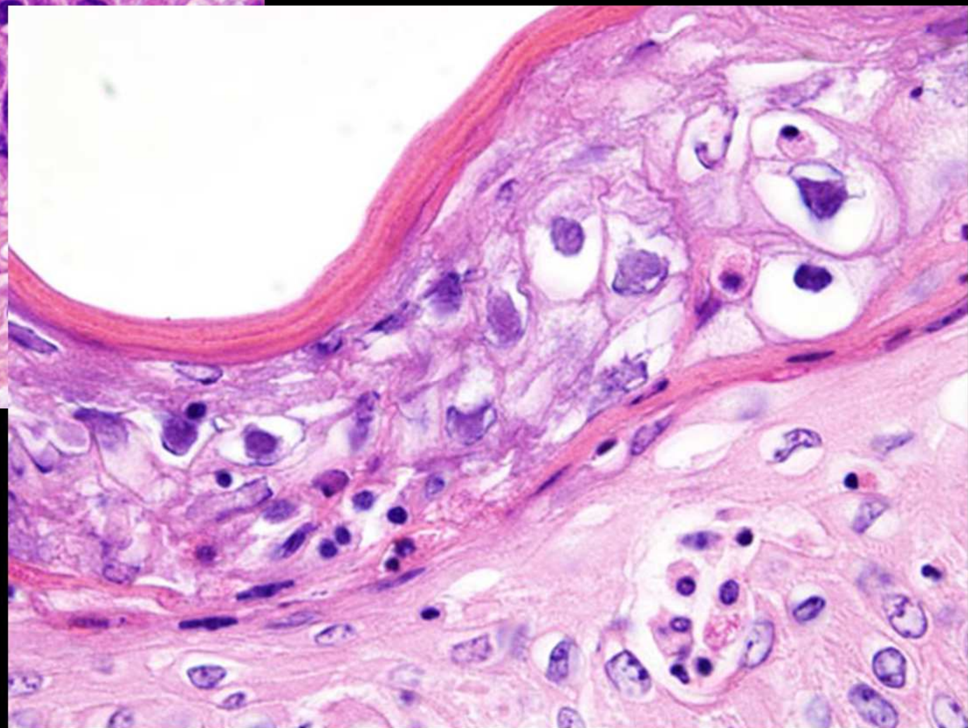

*Keywords:* Loggerhead sea turtle; *Caretta caretta*; Herpesvirus; Loggerhead genital-respiratory herpesvirus; Loggerhead orocutaneous herpesvirus; Chelonivirus

# Genital Lesions in Loggerhead Sea Turtles



# Genital Lesions in Loggerhead Sea Turtles





# ADEN0VIRUS INFECTIONS OF REPTILES

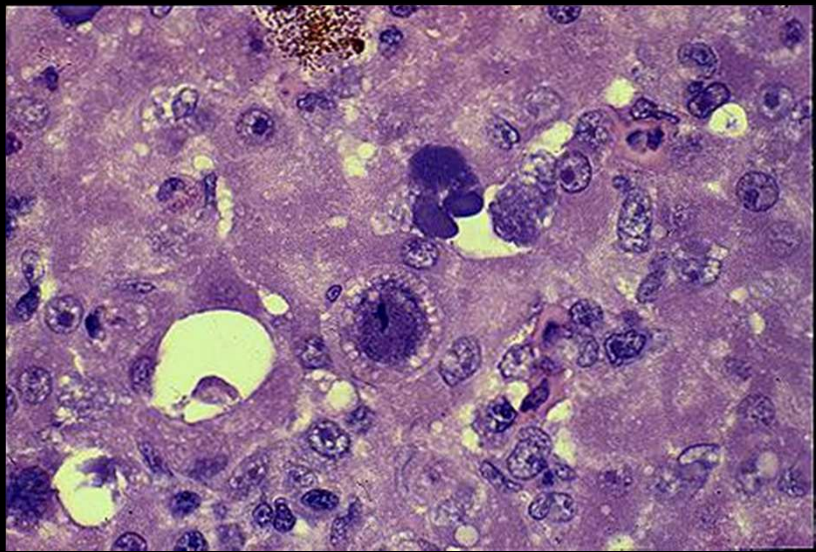
NILE CROCODILE ADENOVIRUS
BEARDED DRAGON ADENOVIRUS
JACKSON CHAMELEON ADENOVIRUS
BOA CONSTRICTOR AND ROSY BOA ADENOVIRUSES
COLUBRID SNAKE ADENOVIRUSES
FORSTEN'S TORTOISE ADENOVIRUS

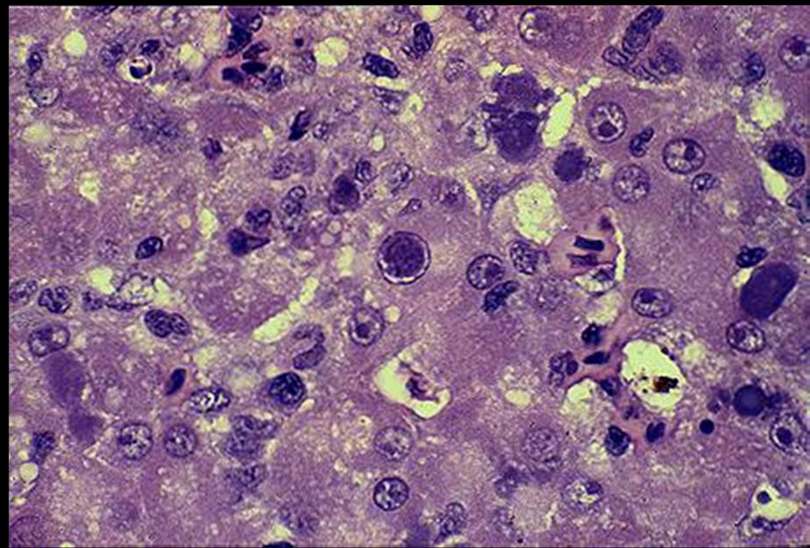
# ADENOVIRUS INFECTIONS OF REPTILES

**Bearded Dragon Adenovirus**

# ADENOVIRUS INFECTIONS OF REPTILES

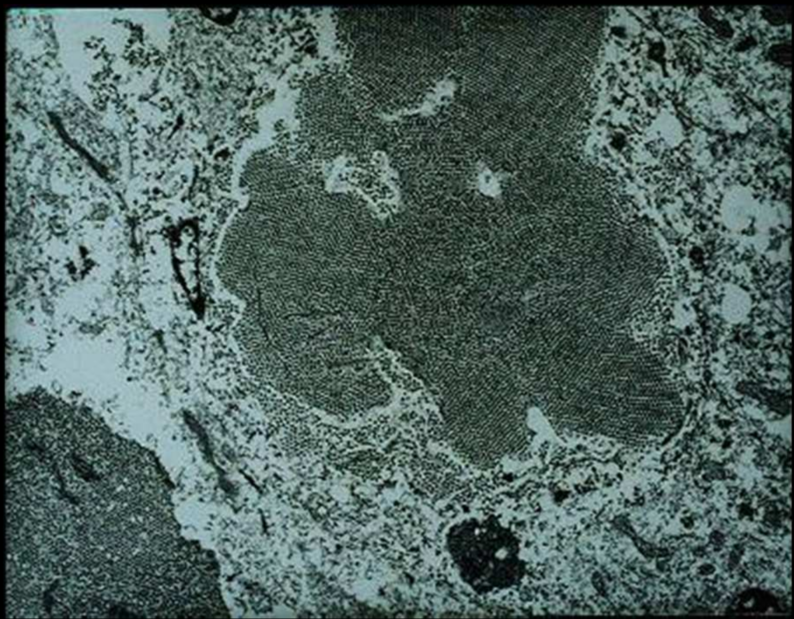
**Bearded Dragon Adenovirus**

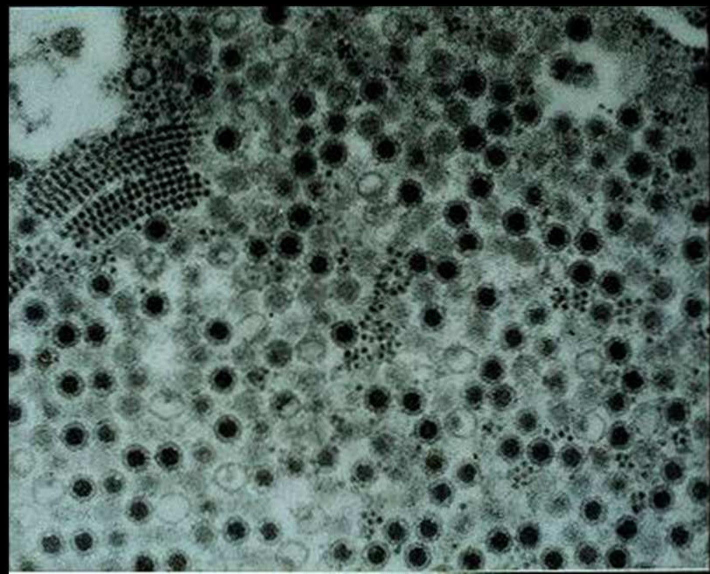




# ADENOVIRUS INFECTIONS OF REPTILES

**Bearded Dragon Adenovirus**





JOURNAL OF VIROLOGY, Dec. 2004, p. 13366–13369 Vol. 78, No. 23
0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.23.13366–13369.2004


# Detection and Analysis of Six Lizard Adenoviruses by Consensus Primer PCR Provides Further Evidence of a Reptilian Origin for the Atadenoviruses

James F. X. Wellehan,[1]* April J. Johnson,[1] Balázs Harrach,[2] Mária Benkö,[2] Allan P. Pessier,[3] Calvin M. Johnson,[4]† Michael M. Garner,[5] April Childress,[1] and Elliott R. Jacobson[1]

*Department of Small Animal Clinical Sciences[1] and Department of Pathobiology,[4] College of Veterinary Medicine, University of Florida, Gainesville, Florida; Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary[2]; Department of Pathology, Center for Reproduction of Endangered Species, Zoological Society of San Diego, San Diego, California[3]; and Northwest ZooPath, Monroe, Washington[5]*

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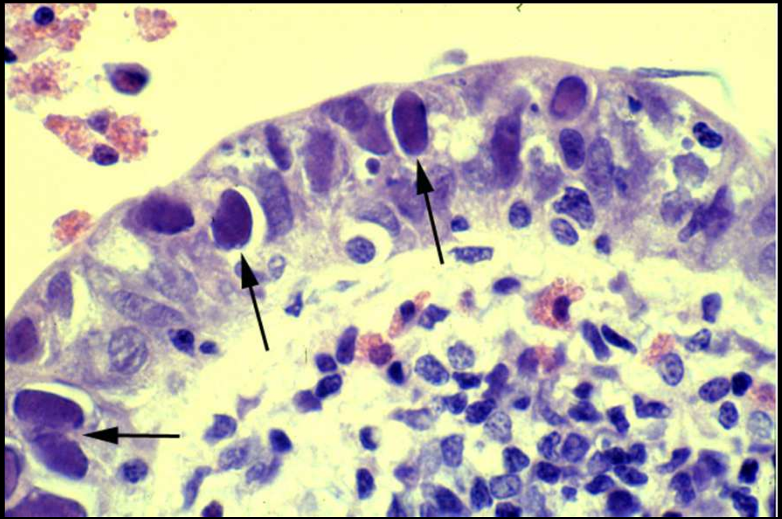
**A consensus nested-PCR method was designed for investigation of the DNA polymerase gene of adenoviruses. Gene fragments were amplified and sequenced from six novel adenoviruses from seven lizard species, including four species from which adenoviruses had not previously been reported. Host species included Gila monster, leopard gecko, fat-tail gecko, blue-tongued skink, Tokay gecko, bearded dragon, and mountain chameleon. This is the first sequence information from lizard adenoviruses. Phylogenetic analysis indicated that these viruses belong to the genus *Atadenovirus*, supporting the reptilian origin of atadenoviruses. This PCR method may be useful for obtaining templates for initial sequencing of novel adenoviruses.**

# Adenovirus Infection of Snakes



# Adenovirus in Forsten's Tortoise, *Indotestudo forstenii*

•A group of 105 Forsten's tortoises (*Indotestudo forstenii*) were confiscated by a government agency.

• Many of the tortoises showed clinical signs of ill health including anorexia, lethargy, mucosal ulcerations and palatine erosions in the oral cavity, nasal and ocular discharge, and diarrhea.

•intranuclear inclusions consistent with an endotheliotropic and epitheliotropic adenovirus were often identified in a large number of tissues, including extensive bone marrow involvement.

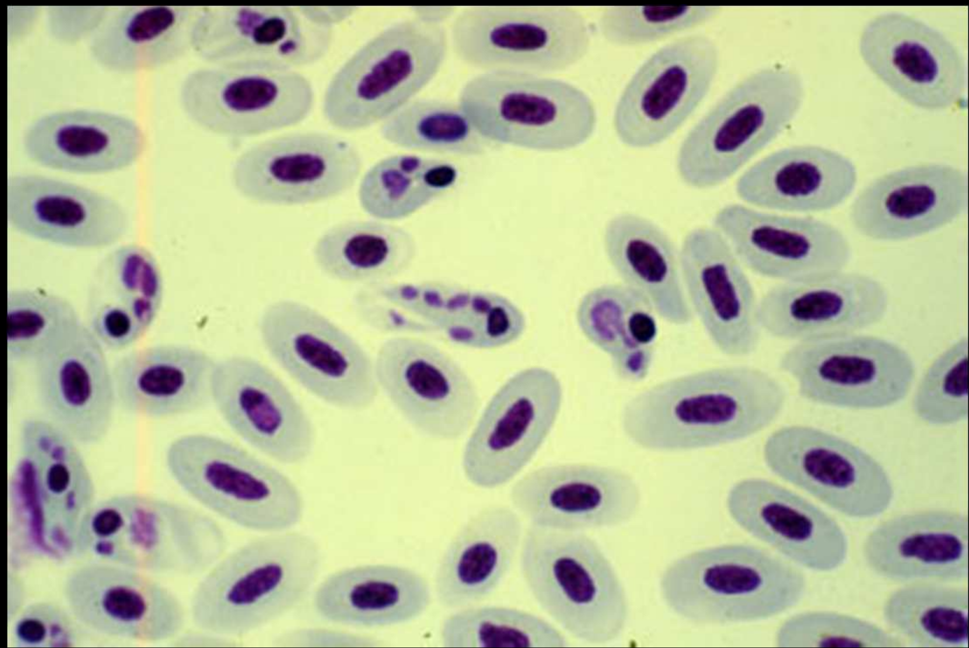
•Two institutions confirmed identical nucleic acid sequences for a novel adenovirus in the genus *Siadenovirus*, which has been submitted to GenBank as Sulawesi tortoise adenovirus 1.

•Presented at the 2007 Zoo and Wildlife Pathology Workshop by Rita McManamon, DVM, University of Georgia.

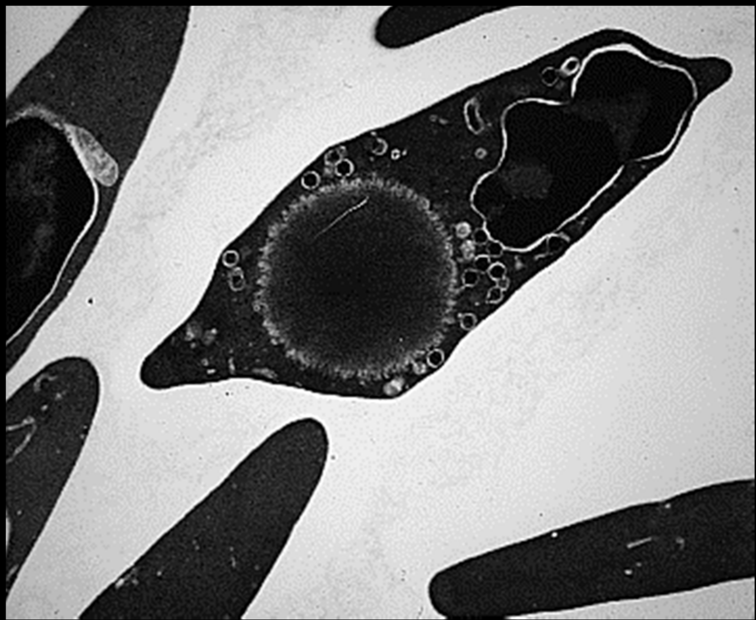
# IRIDOVIRIDAE

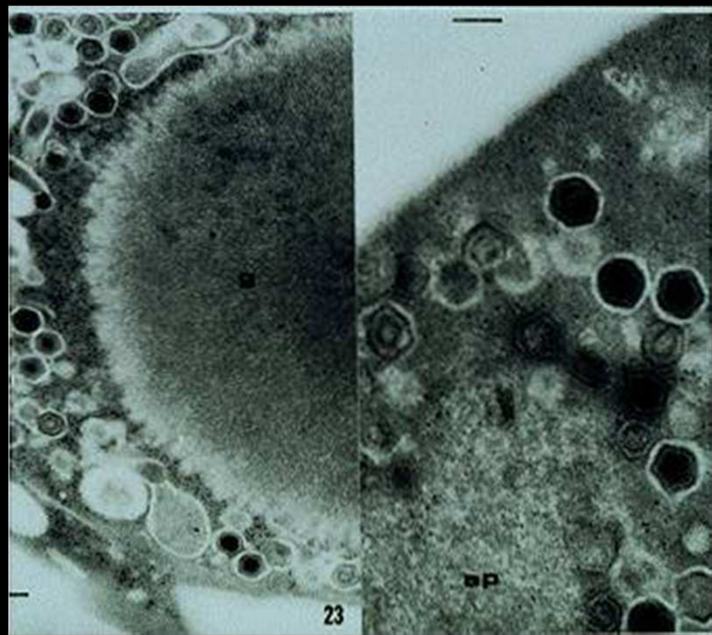
- Pirhemocyton - Lizard Erythrocytic Virus
- Toddia - Snake Erythrocytic Virus
- Iridovirus of Chelonians
- Iridovirus of green tree pythons

# Chameleons - Lizard Erythrocte Virus

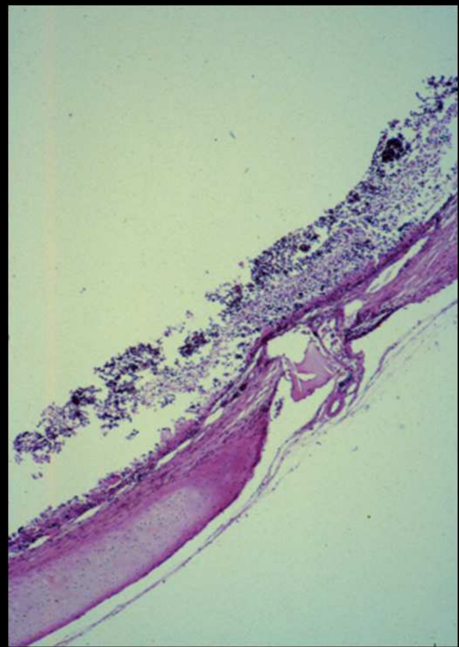


# Chameleons - Lizard Erythrocte Virus





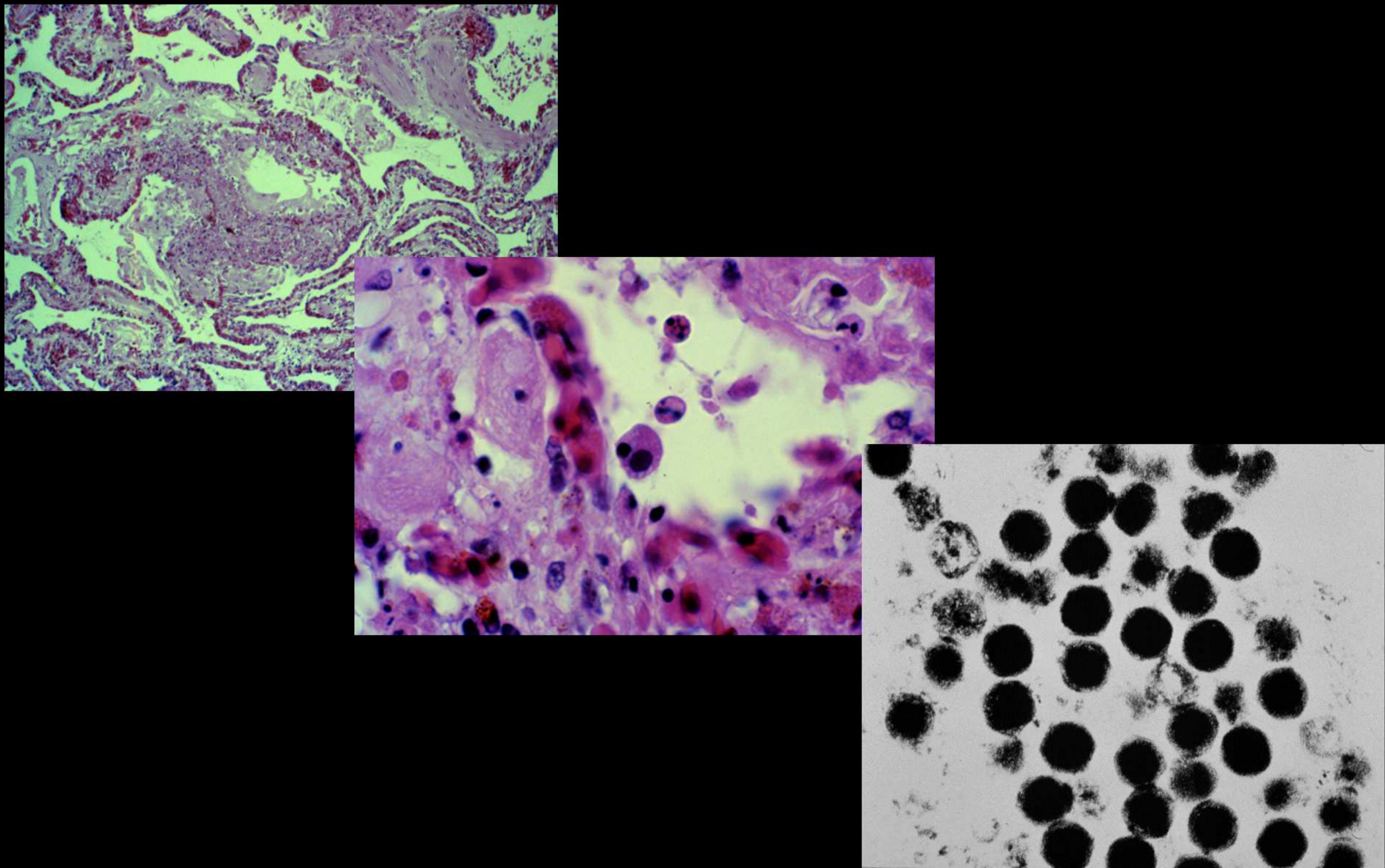
# Iridovirus Infection of Chelonians



Tracheitis

Westhouse, R.K., Jacobson, E.R.,Harris, R.A., Winter, K.R. and Homer, B.L. 1996. Respiratory and pharyngo-esophageal iridovirus infection in a Gopher Tortoise (Gopherus polyphemus. J. Wildl. Dis. 32:682-686.

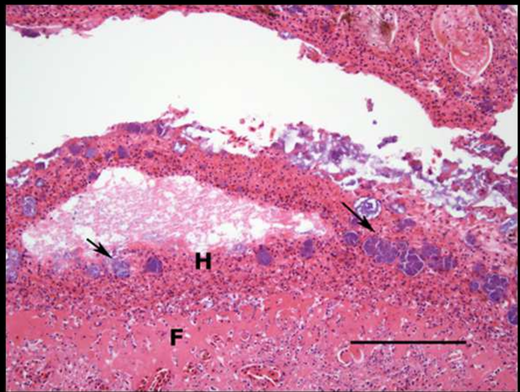
# Iridovirus Infection of Chelonians

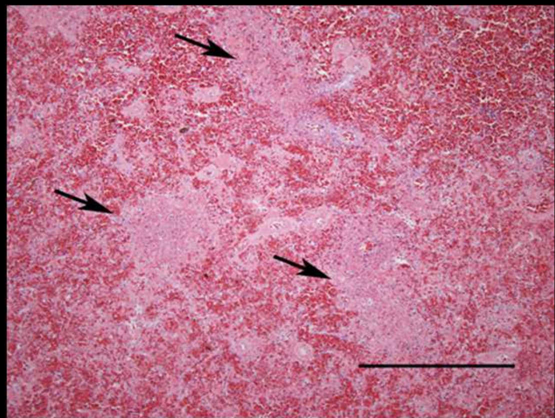


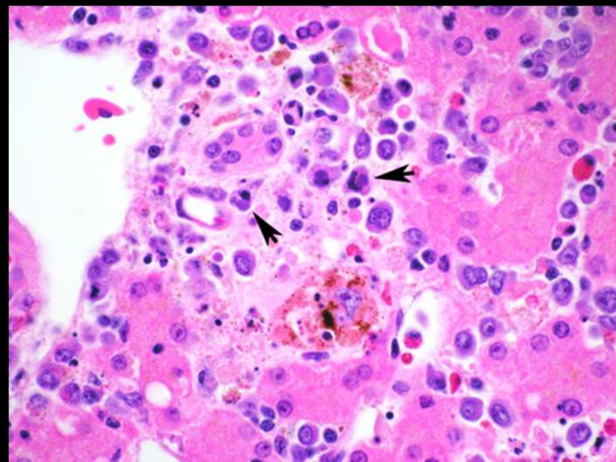
# *Ranavirus* in Box Turtles and Tortoises

# Consistent lesions in infected chelonians:

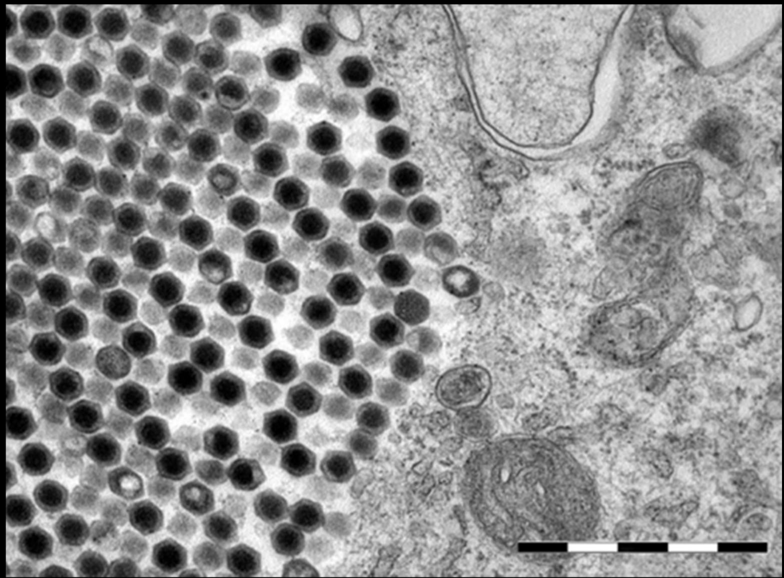
- necrotizing stomatitis and/or esophagitis
- fibrinous and necrotizing splenitis
- multicentric fibrinoid vasculitis
- Intracytoplasmic inclusion bodies rarely observed

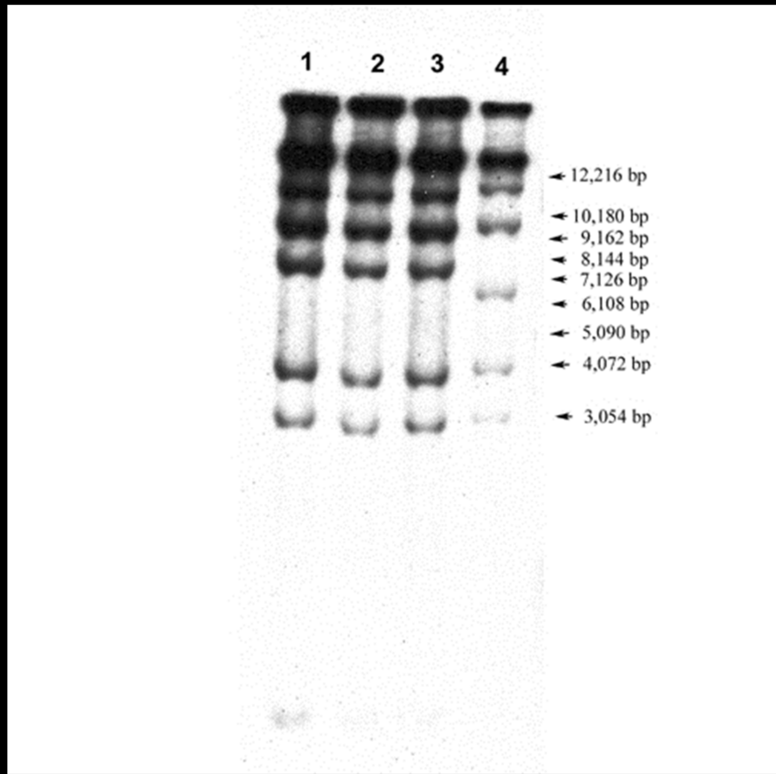




# TEM of *Ranavirus* in tissue section






***Ranavirus*** **DNA radiolabeled with [methyl-$^{3}$H] thymidine and digested with the endonculease *Hind*III. Lanes 1, 2, 3, and 4 represent *Ranavirus* DNA fragments from Frog Virus 3, Burmese star tortoise, Southern leopard frog, and box turtle respectively.**

Vet Pathol 44:285–297 (2007)

# Experimental Transmission and Induction of Ranaviral Disease in Western Ornate Box Turtles (*Terrapene ornata ornata*) and Red-Eared Sliders (*Trachemys scripta elegans*)

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**Abstract.** An experimental transmission study was designed to determine whether a causal relationship exists between a *Ranavirus* (BSTRV) isolated from a Burmese star tortoise that died and the lesions observed in that tortoise. A pilot study was performed with 3 box turtles (*Terrapene ornata ornata*) and 3 red-eared sliders (RESs; *Trachemys scripta elegans*) to assess their suitability in a larger study. Based on the outcome of this study, RESs were selected, and 2 groups of 4 RESs received either an oral (PO) or intramuscular (IM) inoculum containing$10^5$ 50% Tissue Culture Infecting Dose ($TCID_{50}$) of a BSTRV-infected cell lysate. One turtle each was mock inoculated PO or IM with the same volume of uninfected cell lysate. Three of four IM-inoculated RESs developed clinical signs (nasal and ocular discharge [3 of 3], oral plaques [1 of 3], conjunctivitis and hyphema [1 of 3] and extreme lethargy [3 of 3]). A *Ranavirus* was isolated from kidney homogenates of 3 euthanatized turtles; DNA sequences of a portion of the major capsid protein gene were amplified by polymerase chain reaction. Consistent histologic lesions were observed only in IM-inoculated turtles and included fibrinoid vasculitis centered on splenic ellipsoids, multifocal hepatic necrosis, and multicentric fibrin thrombi in a variety of locations, including hepatic sinusoids, glomerular capillary loops, and pulmonary capillaries. Virions compatible with *Ranavirus* were observed within necrotic cells of the spleen of 1 IM-inoculated turtle using transmission electron microscopy. This study fulfills Koch's postulates, confirming a causal relationship between BSTRV and the clinical and histologic changes in chelonians infected with this virus.

# *Ranavirus* Infection

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## FIRST IDENTIFICATION OF A RANAVIRUS FROM GREEN PYTHONS (*CHONDROPYTHON VIRIDIS*)

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ABSTRACT: Ten juvenile green pythons (*Chondropython viridis*) died or were euthanized shortly after having been illegally imported into Australia from Indonesia in 1998. Histologic examination of two of the three snakes that died revealed moderately severe chronic ulceration of the nasal mucosa and focal or periacinar degeneration and necrosis of the liver. In addition there was severe necrotizing inflammation of the pharyngeal submucosa accompanied by numerous macrophages, heterophils, and edema. An iridovirus was isolated in culture from several tissues and characterized by immunohistochemistry, electron microscopy, enzyme-linked immunosorbent Assay, polyacrylamide gel electrophoresis, polymerase chain reaction and sequence analysis, restriction endonuclease digestion, and DNA hybridization. This is the first report of a systemic ranavirus infection in any species of snake and is a new member of the genus, *Ranavirus*.

*Key words:* *Chondropython viridis*, green python, iridoviruses, ranaviruses.