Journal of Herpetological Medicine and Surgery

Ophidian Serpentoviruses: A Review and Perspective

--Manuscript Draft--

Manuscript Number:	JHMS-D-23-00010R1
Article Type:	Review
Keywords:	Nidovirales, pathology, pneumonia, serpentovirus, snake
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Abstract:	Ophidian serpentoviruses, initially referred to as nidoviruses, were first documented in captive pythons nearly ten years ago. Since then, much has been learned about these important pathogens, now classified in subfamily Serpentovirinae of family Tobaniviridae and representing an important emerging pathogen that threatens captive snakes. Serpentoviral infections are best characterized in pythons (family Pythonidae), but have also been documented in boas (family Boidae) and colubrids (family Colubridae), as well as shingleback skinks (Tiliqua rugosa), veiled chameleons (Chamaeleo calyptratus), and the Bellinger River snapping turtle (Myuchelys georgesi). Clinical signs include increased oral mucous secretion, oral mucosal reddening, dyspnea, anorexia, and weight loss. Subclinical infections can also occur, and multiple studies report a lack of correlation between clinical signs and presence of serpentoviral nucleic acids in snakes. Lesions associated with serpentoviral infections predominantly occur in the upper respiratory and gastrointestinal tracts but can also extend to the lungs. Microscopically, these lesions may consist of inflammation, epithelial proliferation, and proliferative interstitial pneumonias, which can be complicated by concurrent bacterial bronchopneumonia. The most common method of diagnosis is reverse transcription PCR to detect viral RNA, and oral/choanal swabs are reliable samples for ante- or postmortem diagnosis. Specific treatment protocols have not yet been described, and management is based on supportive care. This manuscript presents a narrative review of all serpentovirus publications to date with perspective from researchers working to further characterize these pathogens, with the goal of serving as a comprehensive clinical and diagnostic overview for clinicians, zoological curatorial staff, wildlife biologists, and hobbyists.

Ophidian Serpentoviruses: A Review and Perspective

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Abstract

Ophidian serpentoviruses, initially referred to as nidoviruses, were first documented in captive pythons nearly ten years ago. Since then, much has been learned about these important pathogens, now classified in subfamily *Serpentovirinae* of family *Tobaniviridae* and representing an important emerging pathogen that threatens captive snakes. Serpentoviral infections are best characterized in pythons (family *Pythonidae*), but have also been documented in boas (family *Boidae*) and colubrids (family *Colubridae*), as well as shingleback skinks (*Tiliqua rugosa*), veiled chameleons (*Chamaeleo calyptratus*), and the Bellinger River snapping turtle (*Myuchelys georgesi*). Clinical signs include increased oral mucous secretion, oral mucosal reddening, dyspnea, anorexia, and weight loss. Subclinical infections can also occur, and multiple studies report a lack of correlation between clinical signs and presence of serpentoviral nucleic acids in snakes. Lesions associated with serpentoviral infections predominantly occur in the upper respiratory and gastrointestinal tracts but can also extend to the lungs. Microscopically, these

lesions may consist of inflammation, epithelial proliferation, and proliferative interstitial pneumonias, which can be complicated by concurrent bacterial bronchopneumonia. The most common method of diagnosis is reverse transcription PCR to detect viral RNA, and oral/choanal swabs are reliable samples for ante- or postmortem diagnosis. Specific treatment protocols have not yet been described, and management is based on supportive care. This manuscript presents a narrative review of all serpentovirus publications to date with perspective from researchers working to further characterize these pathogens, with the goal of serving as a comprehensive clinical and diagnostic overview for clinicians, zoological curatorial staff, wildlife biologists, and hobbyists.

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Overview

Captive reptiles, particularly pythonid and boid snakes, commonly exhibit signs of oral and respiratory disease. Outbreaks of respiratory disease in captive ball python (*Python regius*) collections in particular have been reported by veterinarians as early as the 1990s (Parrish *et al.*, 2021). Clinical signs of respiratory disease in reptiles can include open-mouthed breathing, altered posture, increased respiratory effort, increased oral secretions and exudation, as well as more generalized, nonspecific signs including weight loss, regurgitation, and anorexia (Dervas *et al.*, 2020). Respiratory disease in reptiles is often due to primary viral etiologies, though secondary bacterial and fungal infections also occur (Hoon-Hanks *et al.*, 2018).

The order *Nidovirales* represents a large group of enveloped, positive-sense, single-stranded RNA viruses (Dervas *et al.*, 2017). Historically, nidoviruses have been shown to infect a wide range of vertebrate and invertebrate hosts. Nidoviruses of clinical veterinary significance

include equine arteritis virus (EAV) in horses, infectious bursal disease virus (IBDV) in chickens, and porcine reproductive and respiratory syndrome virus (PRRSV) in swine (Parrish *et al.*, 2021). Nidoviruses are also of significance to human health, most notably including SARS CoV-2, the cause of COVID-19, and as such represent a group of viruses that has garnered global attention (Parrish *et al.*, 2021). Accordingly, and with the aid of next generation sequencing and modern metagenomic analyses, the size of the order *Nidovirales* continues to expand (Parrish *et al.*, 2021).

The first reptile-associated nidoviruses were discovered in 2014 in multiple collections of captive ball pythons in the United States and a captive Indian python (*Python molurus*) in Germany (Bodewes *et al.*, 2014; Stenglein *et al.*, 2014; Uccellini *et al.*, 2014). Related, yet distinct, viruses were reported soon thereafter in captive green tree pythons (*Morelia viridis*), a Burmese python (*Python bivittatus*), a carpet python (*Morelia spilota*), and boa constrictors (*Boa constrictor*) (Dervas *et al.*, 2017; Marschang and Kolesnik, 2017). Serpentoviruses, as they are most appropriately referred to given an improved understanding of taxonomy and relatedness of this subfamily, have now been described in an array of snake species, primarily in the families Pythonidae and Boidae, but also the Colubridae (Hoon-Hanks *et al.*, 2019; Tillis *et al.*, 2022). Serpentoviruses have also been described in two lizards, the shingleback skink (*Tiliqua rugosa)* and veiled chameleon (*Chamaeleo calyptratus*), as well as one chelonian, the Bellinger River snapping turtle (*Myuchelys georgesi*) (O'Dea *et al.*, 2016; Zhang *et al.*, 2018; Hoon-Hanks *et al.*, 2020). Phylogenetic analysis of these other reptile nidoviruses has shown they are more closely related to serpentoviruses described in snakes than other viruses in the family *Tobaniviridae*, such as bafiniviruses or toroviruses, likely also placing them in the subfamily *Serpentovirinae* (O'Dea *et al.*, 2016; Zhang *et al.*, 2018; Hoon-Hanks *et al.*, 2020).

The goals of this review are to summarize the last decade of research on ophidian serpentoviruses, particularly as it relates to clinical signs of infection, disease pathology, and diagnostic testing options, to inform anyone working with snakes, from clinicians to hobbyists, on this important emerging group of reptile viruses.

Taxonomy and Viral Biology

The viral order *Nidovirales* is characterized by enveloped viruses that have a positive-sense, single-stranded, non-segmented RNA ($ssRNA^+$) genome (Bodewes *et al.*, 2014; Dervas *et al.*, 2017). Several suborders make up the *Nidovirales*, notably including *Cornidovirineae* with family *Coronaviridae, Ronidovirineae* with family *Roniviridae*, *Arnidovirineae* with family *Arteriviridae, Mesnidovirineae* with family *Mesoniviridae*, and *Tornidovirineae* with family *Tobaniviridae* (Bodewes *et al.*, 2014; Zhang *et al.*, 2018; Current ICTV Taxonomy Release, 2023). Coronaviruses and roniviruses are large nidoviruses with 26-33 kb genomes, while arteriviruses are smaller with a 13-16 kb genome. Mesoniviruses are of an intermediate size (Dervas *et al.*, 2017). The families *Arteriviridae, Coronaviridae,* and *Mesoniviridae* all contain viruses known to infect vertebrates, including fish, birds, and mammals, while viruses of the family *Roniviridae* infect crustaceans and insects (Dervas *et al.*, 2017; Latney and Wellehan, 2020; Walker *et al.*, 2022; Current ICTV Taxonomy Release, 2023). The family *Tobaniviridae* is composed of the subfamilies *Serpentovirinae, Piscanivirinae*, *Remotovirinae*, and *Torovirinae*. Viruses of the family *Torovirinae*, including the toroviruses, infect mammals, while viruses of the family *Piscanivirinae*, namely the bafiniviruses, infect ray-finned fishes (Dervas *et al.*, 2017).

While viruses of the order *Nidovirales* have historically been documented to infect insects, crustaceans, fish, birds, and mammals, it was not until the identification of novel viruses in captive ball pythons and an Indian python in 2014 that reptiles were documented as susceptible hosts (Bodewes *et al.,* 2014; Uccellini *et al.,* 2014; Stenglein *et al.,* 2014). Since then, a multitude of snake-associated nidovirus sequences have been reported. To date, all described snake-associated nidoviruses have been classified as members of the novel subfamily *Serpentovirinae* of the family *Tobaniviridae* (Bodewes *et al.,* 2014; Stenglein *et al.,* 2014). Given this classification, these snake viruses are most accurately and colloquially known as serpentoviruses, though the use of the term "nidovirus" is still commonly used by clinicians and individuals in the private sector.

Among snakes, phylogenetic analysis of viral sequences suggests that multiple divergent serpentovirus clades exist (Walker *et al.,* 2022). The International Committee on Taxonomy of Viruses (ICTV) currently recognizes seven ophidian serpentovirus genera and twelve subgenera (Current ICTV Taxonomy Release, 2023). A summary of the current taxonomy for reptile serpentoviruses to be mentioned in this section (where available) is shown in Table 1. The genus *Pregotovirus* comprises many of the commonly seen python viruses including *Ball python nidovirus 1* (BPNV-1) and *Morelia tobanivirus 1* (Morelia viridis nidovirus; MVNV), as well as others documented to infect pythons of the genera *Python, Morelia, Aspidites,* and *Antaresia.* The genus *Septovirus* also contains viruses detected in pythons, including *Septovirus foka* found in a captive reticulated python (*Malayopython reticulatus*) as well as related viruses found in invasive, free-ranging Burmese pythons in south Florida, USA (Tillis *et al.,* 2022). Other ophidian serpentoviruses found in a variety of boa (genera *Corallus* and *Chilabothrus*) and colubrid (genera *Lampropeltis, Lycodon, Myrrophis, Nerodia,* and *Pantherophis*) hosts, represent numerous other viral genera that, interestingly, do not contain any python viruses (Table 1). The genus *Lyctovirus*

contains *Lycodon tobanivirus 1* and other sequences found in semi-aquatic colubrid snakes from China and the United States (Shi *et al.,* 2018; Tillis *et al.,* 2022). The genus *Infratovirus* contains *Infratovirus 1* found in nematodes collected from a Chinese snake species not otherwise identified (Shi *et al.,* 2016). Additionally, the genus *Infratovirus* contains viruses from various colubrids, including *Infratovirus latu* found in a captive Honduran milk snake (*Lampropeltis triangulum hondurensis*), and a related virus found in a free-ranging corn snake (*Pantherophis guttatus*).

The nidoviruses discovered in other reptiles are also most appropriately categorized as serpentoviruses. Two serpentoviruses have been described in veiled chameleons, with *Lyctovirus alpa* (veiled chameleon serpentovirus A) classified in the genus *Lyctovirus,* while *Vebetovirus paba* (veiled chameleon serpentovirus B) is classified in the genus *Vebetovirus.* Both *Berisnavirus 1* (Bellinger River snapping turtle nidovirus) and *Shingleback nidovirus 1* are currently classified as members of the genus *Pregotovirus* (Walker *et al.,* 2022). However, as would be expected for a recently discovered and rapidly growing group of viruses, the taxonomy is in a near constant state of flux and revision. For example, as part of a viral surveillance study of snakes in south Florida, United States, novel serpentoviral sequences identified in samples from invasive Burmese pythons, water snakes, and a corn snake each met threshold measurements as set forth by the ICTV to warrant the creation of three new *Serpentovirinae* genera (Tillis *et al.,* 2022). As novel viral species continue to be discovered and characterized, it is likely more genera and subgenera will be created to encompass the significant viral diversity of the serpentoviruses.

Other Respiratory Viruses of Snakes

As serpentovirus-associated respiratory disease was only first characterized in 2014, a review of serpentoviruses would be remiss without a brief reference to other respiratory viruses of snakes. This is particularly important as it relates to differentiating and distinguishing these pathogens and the disease they cause from each other. Multiple viral families have been associated with clinical respiratory disease in snakes. DNA viruses, including adenoviruses (family *Adenoviridae*), herpesviruses (family *Herpesviridae*), and ranaviruses (family *Iridoviridae*), have all been documented to cause ophidian respiratory disease (as reviewed in Marschang, 2019). Negative-sense, single stranded RNA viruses, including ferlaviruses (family *Paramyxoviridae*), sunvirus (family *Sunviridae*), and reptarenaviruses (family *Arenaviridae*) can all be associated with pulmonary lesions, particularly the ferlaviruses. Lastly, the double-stranded RNA reoviruses (family *Reoviridae*) are another important viral differential for snakes with respiratory disease (Marschang, 2019).

Host Range and Infection Prevalence

Serpentoviruses have been documented in members of the *Pythonidae, Boidae, Colubridae,* and *Homalopsidae* (Hoon-Hanks *et al.,* 2018) as well as the *Viperidae* (unpublished data). Based on scientific reports, serpentovirus infections are most commonly seen in pythons, particularly ball pythons and members of the genus *Morelia* (Marschang and Kolesnik, 2017; Hoon-Hanks *et al.,* 2019Blahak *et al.,* 2020). In a 2016 European study screening for serpentovirus in captive snakes (n = 201), 27.4% of pythons and 2.4% of boas were PCR positive (Marschang and Kolesnik, 2017). The determined serpentovirus prevalence of ~30% in captive boids in Europe was supported by two subsequent studies: a 2020 study that tested samples from 1554 captive boid snakes found 28.2% of samples were positive (Blahak *et al.,* 2020), and a 2021 study of 271 captive pythons

found 29.2% of samples were positive (Racz *et al.,* 2021). In the United States, a 2019 cross-sectional sampling of 639 captive snakes identified serpentoviruses in 26% of the animals tested, most commonly in pythons (nearly 40%) followed by boas (10.1%) (Hoon-Hanks *et al.,* 2019). In the sole study assessing serpentovirus infection rates in free-ranging snakes, overall viral prevalence in invasive Burmese pythons in Florida was 24.4%, with some subpopulations having infection rates as high as 50% (Claunch *et al.,* 2022; Tillis *et al.,* 2022). Though additional research is needed in other snake families, to date serpentoviruses have not been detected in lamprophiids or elapids.

Comparing serpentoviral prevalence studies in the US and Europe shows that the rate of serpentovirus detection was not distributed equally between python species. For ball pythons, the determined average species prevalence across the studies by Hoon-Hanks *et al.* 2019 (5%; n = 136), Marschang and Kolesnik 2017 (22%; n = 408), and Racz *et al.* 2021 (26%; n = 112) was generally less than the total python prevalence (37.7%, 27.4%, and 29.2%, respectively). In contrast, green tree pythons frequently had a higher species prevalence (76%, n = 120, Hoon-Hanks *et al.* 2019; 41%, n = 497, Marschang and Kolesnik 2017; and 24%, n = 67, Racz *et al.* 2021) than the total python prevalence. The higher prevalence in green tree pythons may be significant, but could also relate to the ontogenetic color change of this species, resulting in captive offspring being retained in the breeding colony for longer periods of time prior to being sold. Other species with ontogenetic color changes including carpet pythons and blood pythons (*Python* spp.) with a similar captive market dynamic also often had higher than average viral prevalence, with carpet python prevalence rates of 74% (n = 27), 24% (n = 372), and 55% (n = 31) and blood python rates of 36% (n = 45), 44% (n = 61), and 0% (n = 4), across the three studies, respectively. While not all serpentovirus prevalence studies support these trends (Leineweber and Marschang, 2023),

other factors including differences in sensitivity and specificity between rtPCR protocols or still unknown changes in serpentoviral epidemiology over time may potentially limit comparisons between studies. Regardless of species, the four studies do show a higher prevalence of serpentovirus infection in pythons in comparison to either boas or colubrid snakes. And while ball pythons represent one of the most commonly snakes maintained in captivity, a multitude of other colubrid and boa species are more commonly encountered in the pet trade in comparison to *Morelia* or other *Python* spp., and as such noted prevalence differences are unlikely to be associated solely with the number of animals maintained in captivity.

Studies of viral prevalence in non-pythonid and non-boid snakes are limited. In the study by Hoon-Hanks *et al.*, 2019, of the 116 colubrids tested, only a single Honduran milk snake tested positive for serpentovirus RNA (0.9%). In the 2022 study of free-ranging snakes in Florida by Tillis *et al.*, 208 native colubrid snakes representing 10 genera were screened, and 2.4% (n = 5) were positive, including two brown water snakes (*Nerodia taxispilota*), two Florida green water snakes (*N. floridana*), and one corn snake.

Serpentoviruses have also been discovered in other reptiles, including free-ranging shingleback skinks, free-ranging Bellinger River snapping turtles, and captive veiled chameleons (O'Dea *et al.,* 2016; Zhang *et al.,* 2018; Hoon-Hanks *et al.,* 2020). While the identification of serpentoviruses of both shingleback skinks and Bellinger River snapping turtles occurred in wild animals in Australia, shingleback skink nidovirus-like viruses have been detected in captive *Tiliqua* in North America and Europe (unpublished data; Marschang *et al*., 2020). Much remains to be discovered as it relates to the distribution and diversity of serpentoviruses in both free-ranging and captive reptiles worldwide. For example, a 2022 study analyzing RNA sequence datasets identified novel nidoviruses in samples from a captive slider turtle (*Trachemys scripta*) from

Denmark and a wild Taita blade-horned chameleon (*Kinyongia boehmei*) from Madagascar (Harding *et al.,* 2022).

Risk Factors

Ophidian serpentovirus infection status may correlate to certain environmental or intrinsic risk factors. In the prevalence study by Hoon-Hanks *et al.*, 2019, there was a slight positive correlation found between advanced age and serpentovirus infection, but older age did not increase the likelihood of clinical disease. This was proposed to be a result of increased viral exposure time rather than increased viral susceptibility in otherwise-healthy older snakes (Hoon-Hanks *et al.*, 2019). In the study of invasive Burmese pythons in Florida by Tillis *et al.*, 2022, viral prevalence was found to be higher in male snakes and in snakes with greater mass and snout-vent length. Because older snakes have the chance to grow larger, this trend could similarly be explained by increased exposure time. Male Burmese pythons are also known to gather in large groups during the breeding season in Florida, which could lead to higher viral transmission rates among males (Tillis *et al.,* 2022).

Florida Burmese pythons tested in the fall and winter were also more likely to test positive for serpentovirus (Claunch *et al.,* 2022; Tillis *et al.,* 2022). Because reptiles are ectothermic, the cooler winter months can apply stressors that have an immunosuppressive effect (Tillis *et al.,* 2022). Ectotherm-specific viruses may also replicate better at lower temperatures (Marschang, 2019). However, reptiles have also been anecdotally observed "choosing" a lower temperature when in an advanced state of disease, theoretically to reduce activity level of the inciting pathogen (Perry and Mitchell, 2019). As is true with any infectious disease, the stress of cool weather, as

well as poor husbandry, relocation, handling, or breeding, could have an effect on viral susceptibility (Hoon-Hanks *et al.*, 2019). Further studies are necessary to explore external risk factors that may contribute to serpentovirus susceptibility in snakes.

Clinical Signs and Pathological Findings

Snakes clinical for serpentovirus infection most often present with respiratory signs. Visibly, symptomatic snakes can exhibit excessive oral mucoid secretions (Fig. 1A), open-mouthed breathing, and increased and/or audible respiratory effort (Hoon-Hanks *et al.*, 2018). Other clinical signs can include anorexia, weight loss, decreased body condition score, dysecdysis, or the decreased ability/desire to perch in arboreal species (Parrish *et al.*, 2021). A detailed physical examination may reveal oral, choanal, and/or oropharyngeal mucosal reddening (Fig. 1A), increased mucus or fluid within the oral cavity, stomatitis, nasal discharge, and in severe cases, even tooth loss.

On gross necropsy, lesions are primarily present within the head, proximal esophagus, and lungs. In the head, there can be roughening, reddening, and thickening of the oral mucosa, oropharyngeal mucosa, and the cranial esophagus (Fig. 1B). When complicated by secondary bacterial infections (often gram-negative), there can be mucosal ulceration and diphtheritic plaque formation. Mucoid material, sometimes quite tenacious, can generally be found in the oral cavity and choana, but also occasionally within the trachea and extending down into the lungs (Fig. 1C). In uncomplicated serpentoviral infections, the lungs will often appear wet and heavy with variable thickening of the pulmonary parenchyma (Fig. 1C). With secondary bacterial infections (again, often gram-negative), exudative bronchopneumonia is typical, and the vorbronchus (central lumen

of the boid and pythonid lung) can be occluded by inflammatory exudate with variable amounts of mucus (Figs. 1D and E) (Dervas *et al.,* 2017; Hoon-Hanks *et al.,* 2018; Ossiboff, 2018).

Microscopically, serpentovirus infections are associated with a number of changes in the nasal cavity, oral cavity, cranial esophagus, trachea, and lungs. The most commonly documented changes include epithelial hyperplasia, proliferative interstitial pneumonia, and mixed mononuclear and granulocytic pulmonary inflammation (Fig. 2) (Dervas *et al.,* 2017; Hoon-Hanks *et al.,* 2018; Ossiboff, 2018). Epithelial hyperplasia can be seen in both the trachea and the lungs, with hyperplasia of both type I and type II pneumocytes in the latter (Figs. 2B and C), with variable pulmonary smooth muscle hypertrophy (Hoon-Hanks *et al.,* 2018; Dervas *et al.,* 2020). As proposed by Dervas *et al.* (2020), pneumocyte hyperplasia accompanied by increased mucous secretion and decreased surfactant production thickens the blood-gas barrier, presumably decreasing the efficiency of gas exchange, and eventually resulting in respiratory failure. Pulmonary disease is often substantially worsened by secondary bacterial infections resulting in granulocytic bronchopneumonia (Fig. 2D); faveolar and vorbronchus accumulations of granulocytic and necrotic cellular debris exacerbate decreased pulmonary function. The propensity of serpentovirus positive snakes to have secondary bacterial infections is likely due to viral disruption of both the immune response and physical barriers to infection (Hoon-Hanks *et al.,* 2018).

Microscopic lesions are not restricted to the lung and trachea, however. Epithelial proliferation and mixed primarily mononuclear but also lesser granulocytic inflammation can be seen in the oral mucosa, the oropharyngeal mucosa, the glossal sheath, the nasal mucosa, Jacobson's (vomeronasal) organ, and, notably the cranial esophagus. In snakes, the esophageal epithelium is ciliated and may serve as a site of replication for serpentoviruses (Dervas *et al.,*

2020). Though the lesion is restricted to only the cranial quarter (or shorter) of the esophagus, documenting the lesion is important, as no other described viral respiratory pathogens of snakes cause this change. Though not as frequently documented or observed, viral lesions of the lower gastrointestinal tract have been reported (Dervas *et al.*, 2020). In a subset of the green tree pythons in that study, fibrinonecrotic esophagitis was accompanied by similar lesions in the intestine as well as multifocal vasculitis in the heart, lung, and thymus. The macrophages, endothelial cells, and monocytes surrounding those lesions expressed serpentovirus (nidovirus) nucleoprotein (NP), suggesting potential systemic infection with monocyte-mediated spread (Dervas *et al.*, 2020).

Experimental Infection

The pathogenic potential of serpentoviruses has been confirmed experimentally for BPNV-1 (Hoon-Hanks *et al.*, 2018). Juvenile ball pythons were inoculated with BPNV-1 and clinical signs, including reddening of the oral mucosa and increased mucous secretions, were noted starting four weeks post inoculation. The signs progressed in severity over time and were accompanied by oral petechiation, increased respiratory rate, open-mouthed breathing, and anorexia. Lesions in snakes experimentally exposed to BPNV-1 mirrored those of natural cases with mucinous inflammation of the upper respiratory tract and cranial esophagus, but interstitial pneumonia was only observed in the later stages of the disease in experimental settings (Hoon-Hanks *et al.*, 2018). The latter is an important finding and distinction to make: not all experimentally serpentovirus-infected snakes developed pneumonia, especially in the earliest stages of infection (Hoon-Hanks *et al.*, 2018).

Asymptomatic Infection

Asymptomatic infection with serpentoviruses is not uncommon. In the 2019 Hoon-Hanks *et al*. study, clinical signs were reported to be absent in 41% of pythons and 87.5% of boas testing positive for serpentovirus (Hoon-Hanks *et al.,* 2019). In the 2020 Blahak *et al.* study, 285 of the 439 (64.9%) serpentovirus PCR positive snakes were reported to have displayed no clinical signs, and statistical analysis showed no correlation between clinical signs and serpentovirus PCR detection status (Blahak *et al.,* 2020). The potential for asymptomatic infection seems to increase the more distantly related the detected virus is to the best characterized viruses from ball pythons and snakes in the genus *Morelia*. In the 2022 report by Tillis *et al*. of divergent serpentovirus detection in Burmese pythons, only a subset of the snakes that tested positive exhibited slightly thickened oral secretions and reddening of oral mucosa, and there was no correlation of the mild clinical changes to infection status (Tillis *et al.,* 2022). Divergent serpentoviruses have been detected in multiple species of colubrid snakes, but clinically significant infections have not been reported. Subclinical infections have also been detected with high prevalence in clinically healthy veiled chameleons and shingleback skinks (O'Dea *et al.,* 2016; Hoon-Hanks *et al.*, 2020).

One possible interpretation of subclinical animals that are PCR positive for serpentovirus is samples were collected during periods of viral incubation or re-convalescence in the host (Blahak *et al.,* 2020). It is also possible that positive test results were due to superficial contamination or ingestion of viral particles from the environment, and not indicative of active viral infection (Parrish *et al.,* 2021). However, it is very likely that a subset of serpentovirus positive reptiles represent truly asymptomatic infections. This has been encountered both anecdotally and through molecular diagnostic screening of snake collections by the authors. The potentially high prevalence

of subclinical serpentovirus infections should be considered when designing quarantine or diagnostic screening protocols for captive snakes.

Coinfection

Coinfection by multiple pathogens is a common phenomenon in the pathogenesis of respiratory disease, and ophidian serpentoviral infections are no exception. Secondary bacterial bronchopneumonia is a well-described sequel to serpentoviral infection (Stenglein *et al.,* 2014; Uccellini *et al.,* 2014; Dervas *et al.,* 2017; Hoon-Hanks *et al.*, 2019;). A variety of the usual Gram-negative opportunists, including *Aeromonas*, *Pseudomonas*, *Escherichia*, *Citrobacter*, *Serratia*, and *Providencia* spp., can be involved. This is also true for the oral cavity, where bacterial stomatitis can occasionally mask the underlying viral-associated lesions. The bacterial involvement in cases of ophidian serpentovirus infections can also explain the intermittent and temporary response affected snakes will exhibit to antimicrobial therapy. In snakes with evidence of oral or respiratory disease that recrudesces following discontinuation of antimicrobials, serpentoviral testing may be warranted.

There also appears to be a strong correlation between serpentovirus and *Mycoplasma* detection status. In a study by Racz *et al*. in 2021, nearly 80% of pythons in Europe that were PCR-positive for serpentovirus were also positive for *Mycoplasma*. Similarly, in a smaller study of snakes in Poland, of the 13 serpentovirus PCR-positive snakes, ten (77%) were also *Mycoplasma* positive (Pasterny *et al.*, 2021). Though similar studies have not been performed at scale for snakes elsewhere in the world, a clinically ill blood python with a serpentovirus infection from a Florida collection was also PCR-positive for *Mycoplasma* (Flanders *et al.,* 2021). While only one affected

snake from that collection of blood pythons was included in the above referenced manuscript due to the presence of heterophilic extracellular traps, multiple affected snakes in the colony had both serpentovirosis as well as positive *Mycoplasma* PCR results. Though the pathogenesis of uncomplicated *Mycoplasma* infections in squamates also deserves greater attention itself, there may be synergistic effects of co-infection that may alter morbidity and/or mortality.

Viral co-infections have also been documented. In two studies, green tree pythons were found to have co-infections with a snake retrovirus, though the retrovirus was not considered to be clinically significant, and likely represented an endogenous virus (Dervas *et al.,* 2017; Blahak *et al.,* 2020). Coinfection with multiple, genetically distinct serpentoviruses has been documented in a single blood python (Hoon-Hanks *et al.,* 2019). In that study, two other snakes were suspected to have co-infections. The authors have similarly documented a number of other instances of serpentovirus co-infections in single snake hosts (unpublished data). Viral coinfections have been documented for other snake viruses, such as reptarenaviruses, and such co-infections may have a role in inclusion body disease in boas (Hepojoki *et al.,* 2015; Hetzel *et al.,* 2021). Overall, the potential roles of serpentoviruses and co-infecting pathogens as they relate to the pathogenesis of respiratory disease in snakes warrants further investigation.

Treatment

Serpentovirus infections may be associated with mortality rates of up to 75% in certain susceptible species (Hoon-Hanks *et al.,* 2019). Unfortunately, treatment is based largely on supportive care, including appropriate husbandry and thermal gradients and adequate hydration and nutrition (Perry and Mitchell, 2019). The use of anti-inflammatories, antibiotics,

antiprotozoals, antifungals, and immunomodulators may also be considered, though no standard protocols exist and each animal should be managed on a case-by-case basis (Parrish *et al.*, 2021). To date, no studies have been performed on the effectiveness of specific treatment protocols on serpentovirus infection. Unfortunately, there are few specific antiviral treatment protocols reported for reptiles (Marschang, 2019), and to date no specific serpentoviral protocols have been developed (Parrish *et al.*, 2021), though this is an area of active research.

A non-pharmacologic therapeutic option for snakes with serpentovirus infections that warrants further investigation is temperature. Lower ambient and basking temperatures may serve to both provide a preferred environment for reptile virus replication, as well as causing immunosuppression in ectotherms (Marschang, 2019; Tillis *et al.*, 2022). Conversely, higher temperatures may serve to both increase the host immune response and limit the longevity of serpentovirus in the environment. Though such therapies will likely need to consider thermal ranges of potential host species to ensure that temperature alterations do not create additional stress for the snake, they may offer a readily accessible and economical method to mitigate infection and/or transmission. This is an active area of investigation by our laboratory, and we hope to offer specific comments on this potential option in the future.

Transmission

The route(s) of natural serpentoviral transmission in snakes is an area necessitating further research. In the experimental exposure of ball pythons to BPNV-1 fulfilling Koch's postulates, both oral and intratracheal inoculation resulted in productive infection (Hoon-Hanks *et al.*, 2018). Because viral particles and/or genomic material can be detected in oral secretions,

tracheobronchial lavages, and oral, choanal, and cloacal swabs, multiple routes of transmission, including aerosolization, droplet, fomite, and fecal-oral are possible (Parrish *et al.,* 2021). The potential for vertical transmission has not been exhaustively investigated, although evidence to support such potential hasn't been observed thus far (Hoon-Hanks *et al.*, 2019). However, as serpentoviruses have been detected in tissues other than the respiratory system and proximal gastrointestinal tract, including the oviduct (Dervas *et al.*, 2020), the possibility of vertical transmission should not be excluded without further investigation. The significance of determining potential routes of serpentoviral transmission is indisputable, and additional studies are needed. Given the significance these viruses can pose to not only animal health, but also the economics of captive herpetoculture, clearly identifying where to target the transmission cycle to prevent infection is a critical research objective that must be addressed.

Comparison to Other Ophidian Respiratory Viruses

It is important to distinguish serpentovirus infection from other clinically important respiratory viruses of snakes, including ferlaviruses (paramyxoviruses), sunviruses, reptarenaviruses, and reoviruses.

Paramyxoviruses documented in reptiles are assigned to the genus *Ferlavirus*, and outbreaks have been documented in captive snake collections since 1976 (Marschang, 2019). Severe disease caused by paramyxoviruses usually occurs in viperids, but has been documented in colubrids, elapids, boids, and pythonid snakes. Common clinical signs include opisthotonos, head tremors, hemorrhagic oral exudate, dyspnea, regurgitation, anorexia, and even sudden death. Macro- and microscopically, pulmonary changes are characterized by a proliferative interstitial

pneumonia that can appear similar to serpentovirus infection. However, the finding of hemorrhage, syncytial cells, and cytoplasmic viral inclusion bodies are all unique to ferlavirus infection. Moreover, ferlaviruses can also manifest as inflammation of the central nervous system or coelomic viscera (particularly the pancreas) (Ossiboff, 2018).

Sunviruses (Genus *Sunshinevirus*; Family *Sunviridae*) are associated with varying degrees of neurologic and/or respiratory disease primarily in Australian pythons, including black-headed pythons (*Aspidites melanocephalus*), woma pythons (*Aspidites ramsayi*), spotted pythons (*Antaresia maculosa*), and carpet pythons (*Morelia spilota* ssp. and *Morelia bredli*) (Hyndman *et al.*, 2012). However, there has also been a report of a sunvirus isolated from a ball python in Europe (Marschang *et al.*, 2013). While clinical signs and pathologic findings are most commonly associated with the central nervous system, respiratory lesions characterized by bronchointerstitial pneumonia can occur (Hyndman *et al.*, 2012).

Reptarenaviruses are another important group of snake viruses that includes the causative agent(s) of inclusion body disease (IBD) in boas and pythons. IBD is associated with a wide range of clinical signs, from neurologic signs including torticollis, flaccid paralysis, and opisthotonos to body wasting, anorexia, and regurgitation. While pneumonia can be seen in boas with IBD, it is accompanied by the presence of prominent intracytoplasmic eosinophilic viral inclusion bodies (Schumacher *et al.*, 1994). Moreover, while reptarenavirus infections do occur in pythons, the disease progression is often much more rapid than occurs in boas and the disease is primarily neurologic in nature (Hetzel *et al.*, 2021).

The most important clinical distinction between documented serpentovirus infections and the aforementioned ferlavirus, sunvirus, and reptarenavirus infections is the absence of neurologic signs. Microscopically, the viral inclusion bodies seen in ferlavirus and reptarenavirus infections

are absent in serpentoviral infections. Epithelial syncytia, as seen in ferlavirus infections, are also absent in serpentoviral infections. And no central nervous system inflammation or degeneration has been documented in snakes with uncomplicated serpentoviral infections.

Reptile reoviruses are another important differential for ophidian respiratory disease. Reoviruses have been isolated from outbreaks of respiratory disease in Asian rat (*Orthriophis [Elaphe]* spp.) and corn snakes (Marschang, 2019). Though reoviruses are not as well documented as a cause of respiratory disease in boid snakes, they have been identified in pythons (Ahne *et al.*, 1987; Duncan *et al.*, 2004). While clinically there would potentially be overlap of a reoviral infection causing respiratory disease in boids and serpentoviral infection, histologically ophidian reoviruses have the potential to form epithelial syncytia which are not observed in serpentovirus infections (Duncan *et al.*, 2004).

Diagnostic Testing

The most widely available and reliable method for detecting ophidian serpentoviruses is polymerase chain reaction (PCR) testing. Both conventional and quantitative (real-time) reverse-transcription (qRT-PCR) can be used to detect ophidian serpentoviruses (Parrish *et al.*, 2021). Most PCR assays target the ORF1a or 1b gene, which is the most conserved region of the virus and encodes the polymerase protein (Bodewes *et al.*, 2014). The benefits of PCR include rapid results and relatively high sensitivity and specificity. There are pros and cons to each method of PCR detection for serpentoviruses. qRT-PCR is often more economical due to the elimination of sequence confirmation, and depending on the tested sample, may also be able to provide insight into viral loads. However, as qRT-PCR requires a virus-specific probe in addition to

complementary primers, these assays are able to detect only limited clades of serpentoviruses and as such may miss divergent viruses when used for diagnostic screening. Conventional PCR unfortunately takes longer to complete when amplicons are confirmed by sequencing, and is often more expensive. However, available degenerate primers for serpentoviruses are widely reactive for ophidian viruses, and as such even quite divergent viruses can be detected and ultimately characterized (Hoon-Hanks *et al.,* 2019). Subsequently the tradeoff for increased cost and delay in receiving sample results is the ability to detect a broader range of viruses. Consideration of the clinical scenario, the species to be tested, and the objectives of the investigation are all important to determine the optimal type of PCR testing for each situation.

Recommended antemortem samples include oral/choanal swabs or tracheobronchial lavage (Marschang and Kolesnik, 2017; Hoon-Hanks *et al.,* 2018). While serpentoviruses have also been documented in cloacal swabs and fecal samples, this is likely a result of swallowed mucus and inflammatory debris (Dervas *et al.,* 2020; Parrish *et al.,* 2021). Coupled with the potential PCR inhibitors present in fecal material, cloacal/fecal samples should only be tested as a last resort. Postmortem samples should also include oral/choanal swabs, as well as samples of lung and proximal esophagus (Hoon-Hanks *et al.,* 2018; Parrish *et al.,* 2021). As previously discussed, snakes with mild or early serpentoviral disease may lack pulmonary lesions; as such, oral/choanal swabs are arguably the most reliable sample for detection of serpentovirus in clinical or subclinical snakes either antemortem or postmortem.

While virus isolation *in vitro* is considered the "gold standard" for laboratory diagnosis of many viral diseases, this is not the case for serpentovirus infections. However, some serpentoviruses have been successfully isolated in the laboratory. For example, MVNV was successfully isolated from affected lung tissue inoculated onto green tree python liver and brain

cell cultures (Dervas *et al.,* 2017). BPNV-1 was isolated from an oral swab of an affected ball python inoculated onto diamond python (*Morelia spilota spilota*) heart cells (Hoon-Hanks *et al.,* 2018). However, virus isolation is not a particularly reliable method to find serpentoviruses. The viruses seem to exhibit a preference for certain cell types over others, and attempts to isolate serpentoviruses using available boa constrictor kidney and viper heart cell lines were unsuccessful (Stenglein *et al.,* 2014; Blahak *et al.,* 2020). Establishing cell lines from naturally infected hosts does not always remedy the problematic isolation of these viruses; for example, divergent serpentoviruses in Burmese pythons could not be isolated even on a Burmese python heart cell line established for that purpose (Tillis *et al.,* 2022). While serpentoviral isolation remains an important tool for laboratory research of these viruses, it is not a recommended screening assay.

Both immunohistochemistry (IHC) and *in situ* hybridization (ISH) have been used to successfully colocalize viral nucleic acid and microscopic tissue lesions, and may be helpful for confirmation of serpentoviral-induced disease. Using a polyclonal rabbit antibody targeting the nucleocapsid protein, Dervas *et al.* (2017) demonstrated nidovirus N protein in pulmonary and tracheal epithelial cells. The same study also used ISH to confirm viral nucleic acids within pneumocytes lining faveolar spaces (Dervas *et al.,* 2017). The authors have used ISH designed to specifically target a number of serpentoviruses, and viral nucleic acid is reliably present within oral, tracheal, esophageal, and pulmonary epithelial cells. Extremely strong ISH staining can also be seen within the mucus and cellular debris that accumulates within the oral cavity, esophagus, nasal cavity, and lungs. Though the infectivity of this material remains to be determined, the sheer amount of viral nucleic acid in this material strongly suggests that even small droplets of mucus from infected snakes could potentially be incredibly infectious for susceptible hosts.

To date, no published or commercially available serologic assays exist for serpentoviruses. The development of such assays should be considered a priority for the field. Determination of the antibody response in infected snakes, and how such antibodies may mitigate viral infection may provide key evidence to suggest whether studies assessing vaccination are warranted. While no vaccines have been developed for use in snakes, the significant economic ramifications of serpentovirus infections in large python breeding colonies may justify the time and effort that would likely be needed to develop a serpentoviral vaccine.

Management

As is true for almost all reptile infectious diseases, management of serpentovirus infections in captive snakes is largely dependent on prevention and mitigation of spread. Appropriate and stringent disinfection and quarantine protocols are essential for maintaining the health of captive snake collections. Though studies characterizing the environmental stability and susceptibility to commonly used disinfectants are actively being investigated, data on closely related, surrogate viruses, such as SARS-CoV may provide useful data. SARS-CoV particles can remain infectious in the environment for up to two weeks under optimal conditions, with shorter stability at elevated temperatures and humidity (Chan *et al.*, 2011). Disinfection protocols should aim to eliminate or reduce the pathogen load in enclosures and fomites that may travel between enclosures either on the hands of keepers or on tools or materials transferred between enclosures (Hunt, 2019). This is particularly important given the dense populations of captive snakes that are often housed in tiered, tub-based rack systems in very close relative apposition to neighboring snakes. The most commonly-used disinfectants in veterinary medicine and the herpetoculture hobby are likely effective against enveloped viruses like ophidian serpentoviruses (Hunt, 2019). SARS-CoV2 can

be effectively inactivated by ethanol, sodium hypochlorite, quaternary ammonium compounds, and peroxide at varying concentrations given a contact time of 10 minutes (Lee *et al.,* 2023).

Appropriate quarantine procedures are necessary to prevent the introduction of novel pathogens (or strains of pathogens) to an established collection. Quarantine can also provide a new reptile acquisition the opportunity to acclimate to different husbandry and allow for gradual reduction of the stressors related to packaging, shipping, and/or acclimation (Rivera, 2019). Stress can have an immunosuppressive effect on reptiles generally and has been documented specifically in pythons (Claunch *et al.*, 2022). Quarantine, complete with separate caretakers, equipment, food, and bedding, has been shown to effectively prevent infection rates from increasing in a captive snake collection (Hoon-Hanks *et al.*, 2019). The length of appropriate quarantine will likely vary based on the origin of the snake (captive born versus wild-caught), type of collection (zoological, breeding colony, companion animal), and knowledge of available health history and pre-shipment screening (if applicable). While quarantine recommendations for as short as 14 days for individuals with well-documented health history have been suggested (Rivera, 2019), this is likely too short in most instances to ensure a snake is not subclinical for an infectious disease without repeated, preshipment diagnostic screening for major ophidian pathogens, including serpentoviruses. Moreover, as subclinical serpentovirus infections can be common, an adequate quarantine period is imperative to limit virus transmission within the collection should viral recrudescence occur (Hoon-Hanks *et al.*, 2019). It is the authors' opinion that snake quarantine, particularly for large breeding colonies of substantial conservation, economic, or even sentimental value, should be for a minimum of six months to limit potential disease transmission.

A blanket recommendation for quarantine time for any reptile is generally risky, however, as many factors can play into the risk new acquisitions pose to the existing collection. In addition

to typical considerations, such as whether the snake is captive-bred or wild-caught or if the snake comes from a collection with a documented history of being pathogen-free, other less obvious factors may also warrant attention. For example, in both captive and wild pythons, larger and older animals are more frequently serpentoviral PCR positive, likely the result of greater potential exposure time to the pathogen (Hoon-Hanks *et al.,* 2018; Tillis *et al.*, 2022). As such, adding adult animals to an existing colony may represent a substantially greater risk than the addition of neonates. The makeup of any collection acquiring new animals and the types of snakes acquired should also be considered. A new python being added to a breeding colony of green tree or ball pythons carries much greater risk for the potential of serpentoviral disease transmission than would occur if that same python would be added to a collection primarily composed of colubrid snakes.

Regardless of the situation, diagnostic screening for infectious disease, including serpentoviruses, may be beneficial to reduce the risk of transmission (Rivera, 2019). Serial diagnostic testing of quarantine animals may also be effective at reducing the length of quarantine. For zoological collections or breeding colonies working to cultivate serpentovirus-free populations, the screening practices employed by many zoos to protect against the introduction of the amphibian chytrid fungi, *Batrachochytrium dendrobatidis* and *B. salamandrivorans*, namely three consecutive negative PCR screening tests to clear quarantine (Hyatt *et al.*, 2007; Pessier and Mendelson, 2017), may prove effective. PCR screening is recommended to occur on arrival of the animal in question (unless pre-shipment test results are available), and then at least two subsequent tests at four to six-week intervals. While three negative test results do not eliminate the potential for a non-shedding, subclinically infected snake to go undetected, given experimental and anecdotal evidence of serpentovirus infections becoming most apparent during times of stress (shipping, breeding, changes in ambient temperature), this practice is likely to catch most

serpentovirus infections. However, all pathogens are not created equal, and protocols effective for one infectious agent may be ineffective for another.

Conclusions

This manuscript offers a narrative review of ophidian serpentoviruses through the compilation of reported findings from all publications involving serpentoviruses since their initial discovery in snakes in 2014 in addition to otherwise previously unpublished experiences of the authors. Serpentovirus infections have been best characterized in pythons and boas, but there is very limited characterization in colubrids and viperids and no documentation in other snakes. Serpentoviruses have also been documented in other squamates, including shingleback skinks and veiled chameleons, as well as the Bellinger River snapping turtle. Clinical signs typically include increased oral mucus secretion, heightened respiratory effort, and anorexia. Other nonspecific signs can include weight loss, oral mucosa reddening and upper respiratory inflammation. Pathological changes in the respiratory tract are dominated by an interstitial pneumonia with proliferation of pneumocytes and the respiratory epithelium. PCR screening of oral/choanal swabs offers a minimally invasive and rapid diagnostic option. Treatment is largely centered around supportive care, but prevention and effective quarantine are key.

There are still many unknowns regarding the pathogenesis of serpentoviruses in snakes. Though aerosolized and fecal-oral routes of transmission have been proposed, no definitive studies on viral transmission are published. Increased knowledge regarding transmission methods would likely prove to be essential to develop more specific and effective quarantine and screening protocols. We have very limited knowledge about the potential host range of the major circulating

serpentoviruses in captive animals. And perhaps the greatest unknown is the effect ophidian serpentoviruses may have on wild populations of reptiles. For both the shingleback skink and Bellinger River snapping turtle serpentoviruses, the potential for morbidity and mortality in wild reptile populations is clear. However, serpentoviruses have been detected in free-ranging snakes in North America with no apparent clinical significance to the sampled populations. Moreover, several novel nidoviruses were detected in reptile RNA sequence data, which likely indicates viral diversity in the order *Nidovirales* in reptiles that has yet to be characterized (Harding *et al.*, 2022).

Ultimately, the initial, simultaneous documentation of ophidian serpentoviruses in 2014 followed by numerous other studies documenting the nature and extent of these infections in snakes has highlighted the significance these viruses can pose to the health of snakes. They are a pathogen that all veterinarians treating zoo, exotic, or wildlife species should be familiar with. They are a pathogen that all hobbyists working with captive snakes should be familiar with. And they are a pathogen that all individuals educating veterinary students, veterinary technicians, zoo keepers, and arguably even snake and reptile wildlife biologists should be familiar with. While it would be preferred by all authors of this manuscript that serpentoviruses would fade into the annals of herpetocultural and reptile medicine and disease history, that is unfortunately highly unlikely given our current understanding of these viruses.

Acknowledgements: The authors would like to thank Jim Wellehan, April Childress, Maureen Long and the Florida Veterinary Scholars Program at the University of Florida's College of Veterinary Medicine. MAB received support from Morris Animal Foundation Veterinary Student Scholar Award D20ZO-602. SBT was supported by a Graduate Student Block Award from University of Florida's College of Veterinary Medicine.

Figure Legends

Figure 1. Clinical and gross manifestations of serpentoviral infections. A) A ball python (*Python regius*) with a serpentoviral (*Ball python nidovirus 1* [BPNV-1]) infection, exhibiting increased oral mucoid secretions that are commonly seen in infected pythons. There are also low numbers of multifocal oral petechiae. B) The oral cavity of a ball python (*P. regius*) with a BPNV-1 infection. The oral mucosa is diffusely thickened, with a roughened appearance to the mucosal surface, and widespread mucosal congestion with generalized petechiation. C) Lung and trachea of a ball python (*P. regius*) with a BPNV-1 infection. Within the lumen of the trachea, there is an aggregate of mucus and exudate that nearly occludes the tracheal lumen (arrow). Within the lung, a small amount of mucus and fluid is present within the vorbronchus, but faveoli remain patent throughout. The faveolar septa are diffusely thickened. D) Lung of a ball python (*P. regius*) with a BPNV-1 infection complicated by a secondary gram-negative bacterial infection. The lumen of faveoli and the vorbronchus are extensively obscured or occluded by aggregates of inflammatory debris with some hemorrhage. No culture was performed to identify the bacteria present. E) Lung of a green tree python (*Morelia viridis*) with a serpentovirus infection (novel species, not otherwise characterized). The pulmonary parenchyma is diffusely congested, wet, and heavy. The vorbronchus contains a moderate amount of mucus and exudate.

Figure 2. Varying microscopic severity of *Ball python nidovirus 1* (BPNV-1) associated-pneumonia in ball pythons (*Python regius*). Hematoxylin and eosin. A) Normal lung. Note the presence of faveolar capillaries immediately adjacent to the faveolar lumen. The pneumocytes

overlying faveolar capillaries are essentially inapparent. 600x magnification. B) Mild proliferative interstitial pneumonia. The pulmonary interstitium is mildly expanded and contains low numbers of mononuclear cells, dominated by lymphocytes, and low numbers of granulocytes. Most notably, there is widespread hypertrophy and early hyperplasia of pneumocytes, increasing the distance between faveolar capillaries and the faveolar lumina. Minimal amounts of proteinaceous material and debris are present within faveolar lumina. 600x magnification. C) Moderate to marked proliferative interstitial pneumonia. The pulmonary interstitium is moderately expanded and infiltrated by moderate numbers of granulocytes, and fewer lymphocytes and plasma cells. There is moderate hyperplasia of pneumocytes overlying faveolar capillaries. Faveolar lumina contain small amounts of proteinaceous debris, sloughed epithelial cells, and low numbers of granulocytes. 600x magnification. D) Severe bronchointerstitial pneumonia. The lumen of the faveolus in the image is obscured by large amounts of degenerate cellular debris, granulocytes, and abundant proteinaceous fluid (edema). The surrounding faveolar septa contain markedly congested and ectatic capillaries, surrounded by moderate to high numbers of mononuclear cells and fewer granulocytes. There is multifocal erosion to attenuation of the faveolar pneumocytes in some areas, with hypertrophy and hyperplasia in other areas. Gram-negative bacteria are admixed with the inflammatory infiltrates (Gram stain not shown). 200x magnification.

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Table 1. Current International Committee on Taxonomy of Viruses (ICTV) classification of recognized reptilian serpentoviruses by host, ICTV designation of species, genus, and subgenus, and GenBank accession numbers.

Virus name	Host	ICTV Species	ICTV Genus	ICTV Subgenus	GenBank Accession #
Honduran milksnake serpentovirus	*Lampropeltis triangulum hondurensis*	*Infratovirus latu*	*Infratovirus*	*Selatovirus*	MN161572.1
Chinese snake nematode serpentovirus	Snake-associated nematode, NOS	*Infratovirus 1*	*Infratovirus*	*Xintolivirus*	NC_033700.1
Veiled chameleon serpentovirus A	*Chamaeleo calyptratus*	*Lyctovirus alpa*	*Lyctovirus*	*Chalatovirus*	MT997160.1
Chinese Red-banded snake torovirus	*Lycodon rufozonatus*	*Lycodon tobanivirus 1*	*Lyctovirus*	*Rebatovirus*	MG600030.1
Ball python nidovirus 1	*Python regius*[+]	*Ball python nidovirus 1*	*Pregotovirus*	*Roypretovirus*	KJ541759.1
Morelia viridis nidovirus	*Morelia viridis*[+]	*Morelia tobanivirus 1*	*Pregotovirus*	*Roypretovirus*	MF351889.1
Bellinger River snapping turtle nidovirus	*Myuchelys georgesi*	*Berisnavirus 1*	*Pregotovirus*	*Snaturtovirus*	MF685025.1
Shingleback skink nidovirus	*Tiliqua rugosa*	*Shingleback nidovirus 1*	*Pregotovirus*	*Tilitovirus*	KX184715.1
Reticulated python septovirus	*Malayopython reticulatus*	*Septovirus foka*	*Septovirus*	*Sekatovirus*	MN161566.1
Corallus serpentovirus	*Corallus caninus*	*Sertovirus cona*	*Sertovirus*	*Serecovirus*	MN161561.1
Veiled chameleon serpentovirus B	*Chamaeleo calyptratus*	*Vebetovirus paba*	*Vebetovirus*	*Chabetovirus*	MT997159.1

[+]Also documented in a number of other snake species hosts

Figure 1

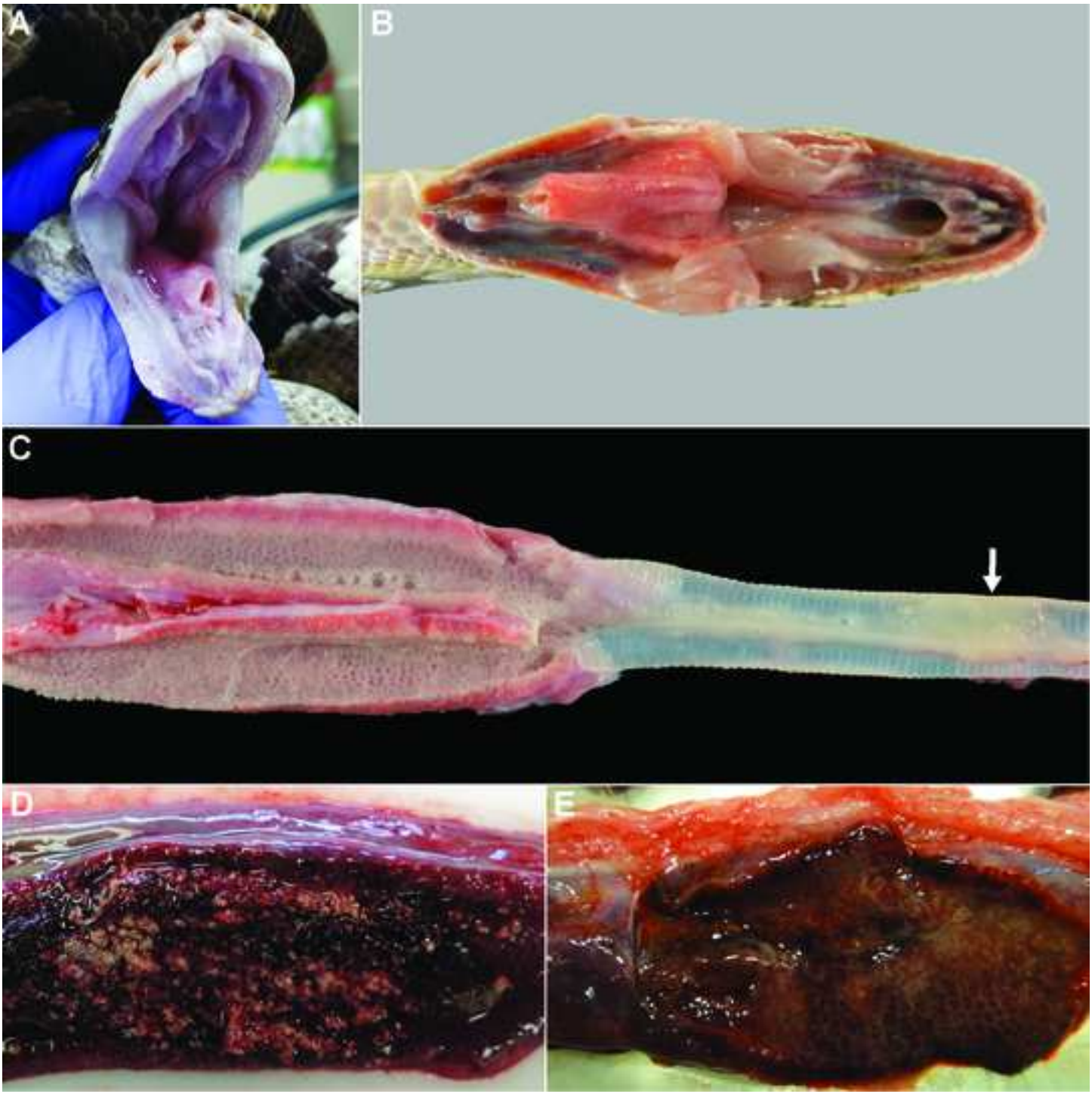

Figure 2