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NOTE

Pathology

A case report of reptile-associated nidovirus (serpentovirus) in a ball python (*Python regius*) in Taiwan

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ABSTRACT. Reptile-associated nidoviruses (serpentoviruses) have been reported to cause proliferative interstitial pneumonia in pythons and other reptile species. A captive, younger than 2 years old, intact female ball python (*Python regius*) showed increased oral mucus, wheezing, and audible breathing with weight loss. Gross and microscopic examination revealed large amounts of mucus in the esophagus and proliferative interstitial pneumonia. Serpentovirus genes were detected from the lung tissues by polymerase chain reaction. The current serpentoviruses was phylogenetically grouped with the serpentovirus previously identified in the US. No case of serpentovirus infection has been reported in Asia. The present report provides information of complete genome sequence and global distribution of serpentovirus.

KEY WORDS: ball python (*Python regius*), nidovirus, respiratory disease, serpentovirus, snake

Nidoviruses are linked to respiratory disease in pythons [2, 11], and the pathogenicity of nidoviruses in pythons has been recently confirmed by an *in vivo* study [3]. In addition to pythons, nidoviruses have been reported to cause proliferative interstitial pneumonia in a variety of animal species, including cattle, lizards, and turtles, suggesting that the nidovirus is potential emerging pathogens warranting further investigation [4, 8, 12]. Phylogenetically, reptile-associated nidoviruses are approximately equidistant from the viruses in the Bafinivirus and Torovirus genuses and presumptively classified into a novel genus [11]. By recent taxonomic research on the order Nidovirales, reptile-associated nidoviruses are now classified within the subfamily Serpentovirinae and named serpentovirus [4].

Cases of serpentovirus infection are documented in Europe and the United States [1, 4, 7]. Reptiles, including pythons, are the most popular exotic pets worldwide, and more than two million reptiles are imported and exported in Europe and the US every year [14]. Therefore, serpentoviruses may have been transmitted to other countries through international trade. However, the global distribution of serpentoviruses is still largely undetermined. We herein report a case of serpentovirus infection in a captive ball python (*Python regius*) in Taiwan, with complete genome sequence of the virus.

A captive, younger than 2 years old, intact female ball python (*Python regius*) was brought from a local commercial reptile breeding facilities in November of 2017 in Taiwan. The python was housed in glass tank with year-round temperature (28 to 30°C) and humidity (50%) control. After approximately 1 year of rearing, the python showed increased oral mucus, wheezing, and audible breathing with weight loss (from 274 g to 200 g) in November of 2018. In 2018, the owner brought 5 new snakes (unknown species) from different countries, and these snakes were housed in the same room but different tanks. The owner announced that none of them showed signs of clinical illness. Symptoms of the python did not improve after antibiotic and antifungal treatments, including 1) enrofloxacin (5 mg/kg, once a day [SID], oral administration [PO]) for 5 days, 2) cephalexin (25 mg/kg, SID, PO) and amikacin (5 mg/kg, every 3 days [Q3D], intramuscular administration [IM]) for 14 days, 3) benzylpenicillin 100,000 IU/kg, procaine benzylpenicillin 100,000 IU/kg, and dihydrostreptomycin sulfate 200 mg/kg (SID, IM) (Shotapen, Virbac, Taipei, Taiwan) with ketoconazole (15 mg/kg, SID, PO) for 7 days, and 4) doxycycline (5 mg/kg, SID, PO) and Bactrim (30 mg/kg, SID, PO) with ketoconazole (15 mg/kg, SID, PO) for 2 days, and the animal was found dead in December of 2018.

Necropsy was performed by referral clinician. At necropsy, large amount of gelatinous to mucoid material was observed in the oral cavity and extended down and occluded the esophagus (Fig. 1A and 1B). The lung parenchyma was mildly thickened, and gelatinous to mucoid material was observed in the cranial portion of lung (Fig. 1C). The amounts of fat bodies were markedly decreased and multifocal mottled pink and tan (Fig. 1D). Formalin-fixed and frozen tissues, including lung and esophagus, were

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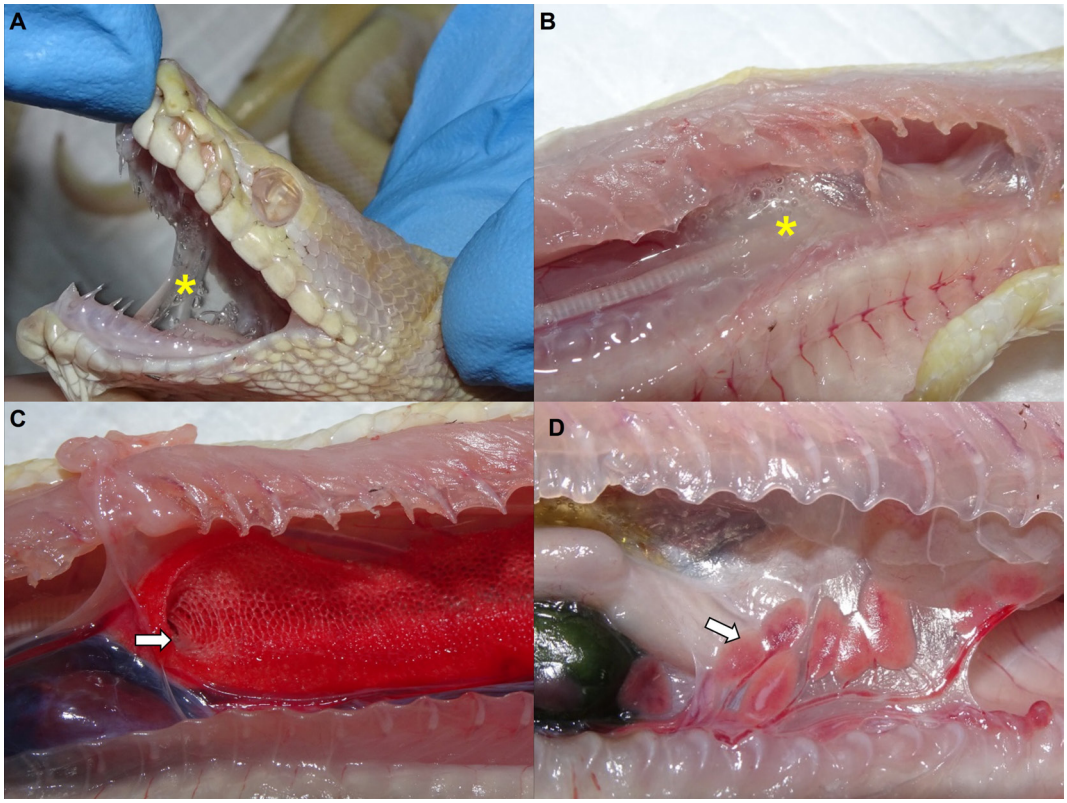

Fig. 1. Macroscopic findings of a captive ball python (*Python regius*). (A) Large amount of gelatinous to mucoid material in the oral cavity (*). (B) Large amount of mucoid opaque material spilling out through the esophagus (*). (C) Mildly thickened pulmonary parenchyma with small amount of mucoid material in the cranial portion (arrow). (D) Markedly reduced amount of fat bodies with pink to red discoloration in caudal coelomic cavity (arrow).

collected for histopathology and molecular diagnosis, respectively. In addition, swab samples from trachea were collected for aerobic bacterial culture.

The formalin-fixed tissues were processed routinely, sectioned at 4 μm and stained with hematoxylin and eosin. Microscopically, some faveoli and central lumens were filled or occluded by a mixture of amorphous eosinophilic substance and mucoid substance with necrotic cell debris. Multifocally, the respiratory epithelium lining the central lumen and septal apices were mild hyperplastic, piling up to more than 10 cell layers thick and forming papillary structures (Fig. 2A). There were low numbers of mixed inflammatory cells, including heterophils, lymphocytes and plasma cells, infiltrating in the faveolar septa (Fig. 2B). The hyperplastic respiratory epithelial cells were swollen and vacuolated with regions of cilia loss and occasional individual cell necrosis, characterized by pyknotic nuclei and shrunk, hypereosinophilic cytoplasm (Fig. 2C). In esophagus, a mixture of amorphous eosinophilic substance, mucoid substance, and low amounts of necrotic cell debris occluded the lumen (Fig. 2D). Based on the gross and microscopic findings, the primary differential diagnosis was serpentovirus infection. However, other etiologies including toxic gases, ophidian paramyxovirus (OPMV), and bacterial infection such as mycoplasma could not be ruled out [3].

DNA and RNA were extracted from the frozen lung tissues and used in polymerase chain reaction with primer sets targeting the genes of serpentovirus [13], OPMV [9], and *Mycoplasma* spp. [10]. Positive controls for OPMV and *Mycoplasma* spp. prepared from positive samples of previous cases were included in both PCR tests. No positive control for serpentovirus was used since there was no known case of serpentovirus infection in Taiwan. The lung tissues were positive for serpentovirus but negative for OPMV and *Mycoplasma* spp. No bacteria were isolated from the trachea by aerobic bacterial culture.

For amplification and analysis of the whole genome of present serpentovirus, seven pairs of primers that covered the overlapping fragments of the genome were used [13]. PCR products were purified and cloned into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Recombinant plasmids from 10 to 20 clones with the amplified amplicons from each PCR reaction were purified using the QIAprep Spin miniprep kit (Qiagen, Valencia, CA, USA) and sequenced in both directions with primers flanking the inserts. The sequences of present serpentovirus were aligned and compared to those of serpentovirus from different geographic locations by MEGA X [5]. The serpentovirus genome from this case was 33,483 nucleotides and 92.7% identical to that from ball pythons in the US (GenBank accession no. KJ541759) with putative ribosomal frameshift signal (RFS) sequence near the end of open reading frame (ORF) 1a [11]. Furthermore, the amino acid sequences of full-length ORF-1a and 1b were used for phylogenetic analysis [16]. The current serpentovirus was grouped with the serpentovirus identified in ball pythons (KJ541759 and MG752895) and a woma python (*Aspidites ramsayi*) (MN161571) in the US by the

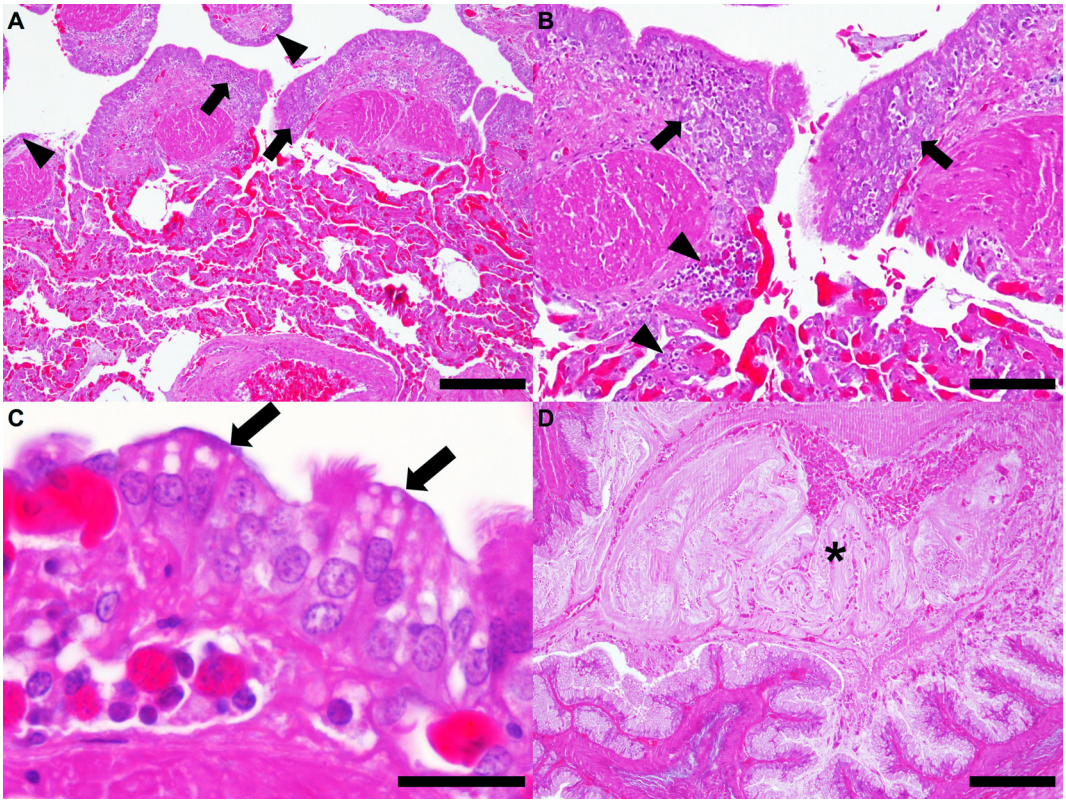

Fig. 2. Microscopic findings of a captive ball python (*Python regius*). (A) Variably hyperplastic respiratory epithelium lining the septal apices (arrows). Note the non-hyperplastic respiratory epithelium (arrowheads). Hematoxylin and eosin (HE) stain. Bar=200 μm. (B) Hyperplastic respiratory epithelium piling up to more than 10 cell layers thick (arrows) and inflammatory infiltrates expanding the faveolar septa (arrowheads). HE stain. Bar=80 μm. (C) Swollen and vacuolated respiratory epithelial cells with regions of cilia loss (arrows). HE stain. Bar=20 μm. (D) A mixture of amorphous eosinophilic substance, mucoid substance, and low amounts of necrotic cell debris occluding the lumen of esophagus (*). HE stain. Bar=200 μm.

phylogenetic tree based on the amino acid sequence of ORF 1a and 1b (Fig. 3) [3, 4, 11].

Previous studies conducted in Europe and the US have demonstrated that serpentovirus can be detected by PCR using oral swabs or blood samples from several snake species, including pythons (Pythonidae family), Boids (Boidae family), and Colubrids (Colubridae family), with or without documented respiratory diseases [4, 7]. Characteristic postmortem findings in pythons with serpentovirus infection included stomatitis, sinusitis, pharyngitis, tracheitis, esophagitis, and proliferative interstitial pneumonia, and there were markedly increased mucus secretion in oral cavity, esophagus, trachea, and lung [3, 4]. Additionally, secondary bacterial infections were also common findings in clinically affected pythons [3]. In this case, the ball python had large amounts of mucus in the oral cavity and esophagus with mild proliferative interstitial pneumonia. The macroscopic and microscopic findings coupled with PCR results confirms the diagnosis of serpentovirus infection.

Since Taiwan is an island country, the transmission route of serpentovirus is presumptively through the import of pet reptiles. According to the record from Taiwan government (https://www.conservation.forest.gov.tw/0001296), more than 300,000 exotic reptiles, including snakes and pythons, have been imported into Taiwan from different countries all over the world during 2001 and 2010. In our case, the current ball python was bred in a local reptile breeding facility, but the owner refuse to provide any further information on the source countries of other pythons. Although the serpentovirus isolated in this case is most similar to that in the US, a definite source and transmission rout of serpentovirus cannot be confirmed. However, the current case, at least, demonstrates that serpentovirus have invaded Taiwan, and the other countries in Asia might be under the same risk as well.

Most importantly, some of the imported reptiles may escape or be intentionally released from captivity (due to prayer animal release) and become invasive species in Taiwan [6]. Invasive species are considered one of the most important threats to global biodiversity, and the endemic species may be negatively affected by the invasive species due to competition on habitat and food, hybridization, and introduction of exotic infectious pathogens [6]. In Taiwan, more than 50 different snake species have been documented. Although Burmese pythons (*Python molurus bivittatus*) is the only python species endemic in Taiwan, snakes within the Colubridae family are common wild snakes [15]. A previous study has indicated that colubrid snakes can be infected by serpentovirus [4], and thus the negative impact caused by serpentovirus to wild snake populations endemic in Taiwan should not be over looked. Further investigation is warranted to determine any potential negative impacts caused by serpentovirus infections in exotic snakes and wild snake populations.

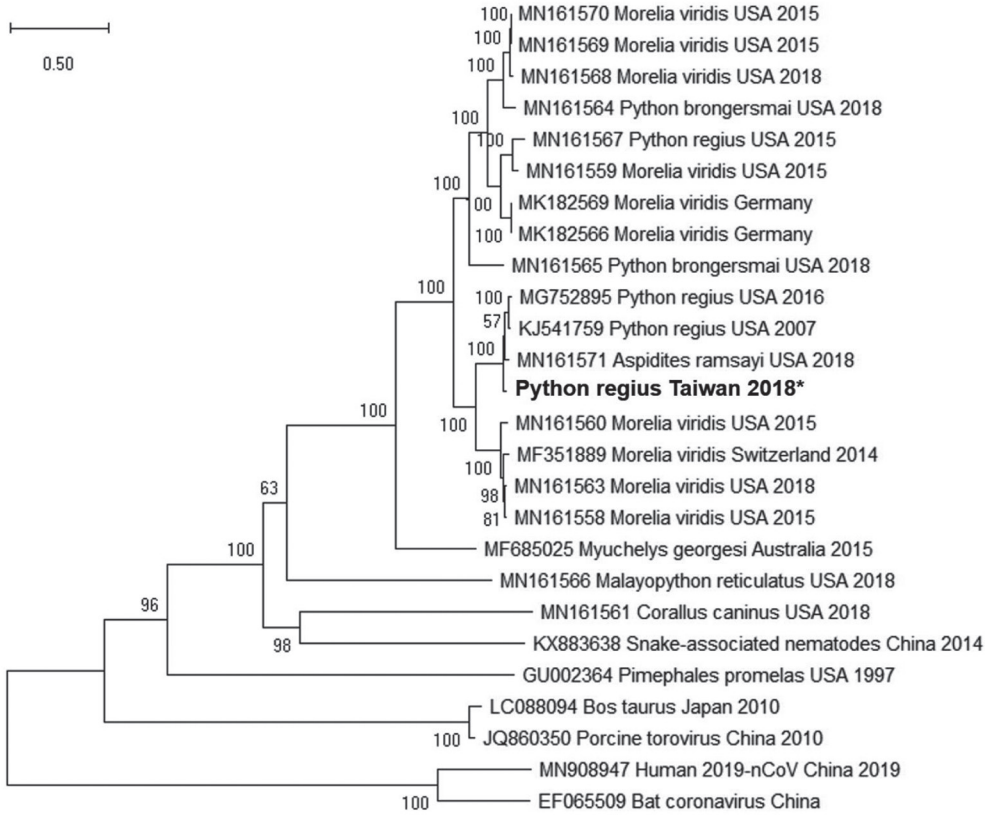

Fig. 3. Phylogenetic tree based on the amino acid sequence of full-length open reading frame (ORF) 1a and 1b of serpentovirus from ball python in Taiwan (*) and other serpentovirus. Analysis was conducted using the Maximum Likelihood method based on the JTT matrix-based model with 1,000 bootstrap replicates (MEGA X). Branch lengths are indicated by scale bar. The percentage bootstrap value associated with each lineage is indicated.

CONFLICT OF INTERESTS. No potential conflict of interest was reported by the authors.

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