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NOVEL POXVIRAL INFECTION IN THREE FINCH SPECIES ILLEGALLY IMPORTED INTO TRINIDAD, WEST INDIES, WITH IMPLICATIONS FOR NATIVE BIRDS

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Abstract: *Oryzoborus angolensis* (Lesser Seed-Finch), *Oryzoborus crassirostris* (Large-billed Seed-Finch), and *Sporophila intermedia* (Grey Seedeater) are finch species native to the Caribbean island of Trinidad. These species are locally trapped and kept for their song, but with declining native populations, enthusiasts have turned to illegally importing birds from the South American mainland. The smuggling of wild birds from South America poses significant disease risks to the native bird species of Trinidad. Herein we describe the first case of poxviral infection in these illegally imported birds in Trinidad and partial genome sequence of the causative agent. Phylogenetic analysis of the 4b core protein sequence indicated that the avian poxvirus identified was most closely related to a 2012 avian pox sequence from Brazil, with 96.2% and 98.1% identity at the nucleotide and amino acid level.

Key words: Finches, 4b core protein, poxvirus, sequence analysis, Trinidad.

INTRODUCTION

Oryzoborus angolensis (Lesser Seed-Finch; Bullfinch), *Oryzoborus crassirostris* (Large-billed Seed-Finch), and *Sporophila intermedia* (Grey Seedeater; Picoplat) are finch species native to the Caribbean island of Trinidad.⁵ They are highly favored by bird fanciers in Trinidad for their singing ability, which has led to a high demand for captive birds. The large volume of capture and possibly anthropogenic factors, such as pesticide use and habitat encroachment, over the years have likely led to population declines. These three species are now found rarely in the wild in Trinidad,⁵ which has resulted in their illegal importation from Venezuela and Guyana on the nearby South American mainland. Anecdotal evidence has suggested that these smuggled South American birds are phenotypically distinct (in song and morphology) from

the native finches in Trinidad. When illegally imported birds are seized by state authorities and after the conclusion of legal proceedings, the courts often decree the release of the birds into the wild. The birds are often released into habitats where native birds may still be found in Trinidad. The disease status of these imported birds is largely unknown, and having undergone the stress associated with transit from the mainland under poor environmental conditions (i.e., small overcrowded cages and lack of food and water), the birds could be more prone to shedding disease pathogens if present. Many subclinical pathogens that may not have had a significant effect on wild bird populations in South America may be detrimental to the naive local finch populations in Trinidad that are already facing population declines. To date, no studies have been conducted to determine the disease risk posed from birds illegally imported into Trinidad.

Avian poxviruses (avipoxviruses) belong to the genus *Avipoxvirus* in the *Poxviridae* family. They typically produce two forms of disease in birds: the dry/cutaneous form, which manifests mainly in unfeathered areas, and the wet/diphtheritic form, which manifests as a necrotic caseous membrane in the upper respiratory tract, mouth, and pharynx.^{1,12} A viremic form has also been described that produces high mortality.^{14,17}

The International Committee on Taxonomy of Viruses (www.ictvonline.org) currently recognizes 10 species of Avipoxviruses (*Fowlpox virus*, *Canarypox virus*, *Juncopox virus*, *Mynahpox virus*, *Psittaci-*

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nepox virus, *Sparrowpox virus*, *Starlingpox virus*, *Pigeonpox virus*, *Turkeypox virus*, and *Quailpox virus*) that are generally host species specific.¹⁸ However, new isolates that may represent new species, strains, or variants of avipoxviruses continue to be identified in other avian species.⁷ Phylogenetic analysis indicates that species fall into three main clades (i.e., clade A [fowlpox clade], clade B [canarypox clade], and clade C [*Psittacinepox virus* clade]).⁷

Avian poxviral infection was previously reported in four species of birds in Trinidad; the golden-headed manakin (*Pipra erythrocephala*), the white-bearded manakin (*Manacus manacus*), the voilaceous euphonia (*Euphonia violacea*), and the bare-eyed thrush (*Turdus nudigenis*).¹⁵ Within the region, the virus was detected in *Sporophila* sp. in Panama;⁸ however, to the best of our knowledge, to date there have been no reports of poxviral infection in *O. angolensis*, *S. intermedia*, and *O. crassirostris*. Avipoxviruses are thought to play a role in declining wild bird populations and can pose a challenge to conservation efforts for threatened species.^{1,2} Poor flight ability, reduced endurance, ataxia, and weakness associated with poxviral infection probably contribute to increased susceptibility to predation.¹⁴ Wild bird populations on isolated islands generally succumb to higher levels of morbidity and mortality, and higher prevalence levels of poxviral infections are observed in warmer areas.¹⁸ Additionally, it is thought that native birds are more significantly impacted by the disease than introduced species.¹⁹ For example, avian pox had a negative effect on the population of native birds in Hawaii after the introduction of the mosquito and exotic domestic birds in the 1800s.¹⁹ Likewise, an introduced poxvirus could have a devastating effect on native populations of birds in Trinidad, such as *O. angolensis*, *O. crassirostris*, *S. intermedia*, or other closely related species in which the virus has not been previously recorded.

CASE REPORTS

Sampling

During the period May 2008 to January 2017, four *O. angolensis*, five *S. intermedia*, and one *O. crassirostris* were presented either as live patients to the Veterinary Teaching Hospital (VTH) of the School of Veterinary Medicine (SVM), University of the West Indies, or for necropsy at either the Pathology Unit of the SVM or the Veterinary Diagnostic Laboratory (VDL), Ministry of Agriculture, Lands, and Fisheries. They were all



Figure 1. Carcass of *Oryzoborus angolensis* with raised lesions around the eyelids and on the leg extending from the digits to the hock.

suspected to be illegally imported from South America, and some had been sold to private pet owners or pet shop proprietors for resale. Illegally imported birds are regularly seized by the local wildlife authorities from smugglers at the point of entry or from pet shops at the point of sale.

The first *O. angolensis* (OA1) was brought to the VDL for necropsy in May 2008. It was a captive adult male bird that was wild caught as a juvenile and raised for 5 yr in captivity. During this period, there were no significant health problems until it developed “pox-like” lesions and then died shortly after. The owner had 12 other birds of the same species at the time and indicated that the birds were bought and sold on a regular basis. None of the other birds were apparently affected.

The second *O. angolensis* (OA2) was a juvenile male that was bought by a pet shop proprietor in September 2008 as part of a consignment of 20 birds acquired for resale. Within a week, the bird developed small eyelid and tarsal lesions and was seen by a private veterinary practitioner 3 days after. A topical ophthalmic oxytetracycline ointment (Terramycin®, 3.5 g, Delegación Sta Ana, Tlapaltitlán, 50160, Toluca, Mexico) was prescribed for the secondary keratoconjunctivitis. Following 5 days of this treatment, the bird was brought to the VTH, SVM, with the chief complaints of anorexia and inability to fly. On clinical examination, it was found to have multiple, proliferative, coalescing, and necrotic lesions in periorbital and tarso-metatarsal areas. The ulcerated lesions were severely hemorrhagic (Fig. 1), and a decision was taken to euthanize the bird on humane grounds.

The third *O. angolensis* (OA3) was a juvenile male purchased in October 2010 that developed bilateral eyelid swellings soon after purchase. The swellings progressively increased in size over the period of 1 wk. The bird was then taken to a

private veterinary practitioner who prescribed topical ophthalmic oxytetracycline and Polymyxin B sulfate ointment therapy (Terramycin, 3.5 g, Delegación Sta Ana). The lesions were unresponsive to the treatment, and 3 days later, the bird became anorexic and was unable to move in the cage. The bird died 2 days later, and the owner brought it to the SVM for necropsy.

In January 2017, the fourth *O. angolensis* (OA4), a juvenile female, was bought by a pet shop owner as part of a consignment of 10 birds for resale. It also developed a swelling on the left eyelid that progressively increased in size. It became anorexic for 2 days and was unable to move in the cage. Following this, it was brought to the VTH, SVM. The bird was euthanized following clinical examination.

An illegally imported consignment of 37 *S. intermedia* was seized by wildlife authorities in May 2014, with significant mortality (14/37) in overcrowded cages. In seven of the remaining 23 birds, multiple, variably sized (2–6 mm in diameter), proliferative, crusting, and necrotic nodular swellings were detected on the eyelids and the commissures of the beak. These seven symptomatic birds were brought to the VTH, SVM, for treatment. Five of these (SP1–5: two juvenile males, two juvenile females, and one adult male) had severe lesions, were unable to move in the cage, and were anorexic for over 2 days. Following clinical examination, these five birds were euthanized. The remaining two birds had a single nodule on an eyelid and tarsus, respectively, both less than 2 mm in diameter. They were placed in individual cages and treated with trimethoprim sulfamethoxazole (400 mg/L drinking water) and given daily multivitamins (Miltivit CH, KELA NV, St. Lenaartseweg, 48, 2320, Hoogstraten, Belgium). In approximately 2 wk, the lesions regressed, and the birds survived. In June 2016, another consignment of seven *O. crassirostris* was seized by wildlife authorities. These birds were submitted to the VDL, and at that time, one bird was found dead at the bottom of the cage and the other six were euthanized. The dead bird (OC1), a juvenile male, was the only bird that exhibited proliferative, 2–4-mm-diameter nodules, which were located around the nares. The other birds were grossly normal.

A necropsy was performed on each bird, and tissues, including lesions on the eyelids and nares and commissures of the beak, tarsus, lungs, liver, and kidneys, were harvested and fixed in 10% buffered formalin for 48 hr. Fresh tissues exhibiting lesions were frozen at -80°C .

Histology

The formalin-fixed tissues were embedded in paraffin, cut in 4- μm sections, and prepared for staining with hematoxylin and eosin.

Electron Microscopy

Electron microscopy was performed on samples of formalin-fixed paraffin embedded pox lesions from one *O. angolensis* bird (OA3). The paraffin blocks were deparaffinated, postfixed in osmium tetroxide, and embedded in epoxy resin before thin sections were cut and viewed with a JEM-1210 transmission electron microscope (JEOL, Tokyo, Japan).

Polymerase Chain Reaction and Sequencing

DNA was extracted from fresh tissue of one bird for each of the three species exhibiting typical pox-like lesions (i.e., OA4, SP3, and OC1) using the DNeasy Blood and Tissue Kit (QIAGEN Inc, 27220 Turnberry Lane, Suite 200, Valencia, CA 91355, USA) following the manufacturer's instructions. Polymerase chain reaction (PCR) was used to amplify a 578-bp fragment of the avipoxvirus 4b core protein gene as previously described by Lee and Lee, using primers CP1–5' CAGCAGGTGCTAAACAACAA–3' and CP2–5' CGGTAGCTTAACGCCGAATA–3'.⁹ Amplification was confirmed visually under UV light by the presence of an appropriately sized band (~580 bp) on an agarose gel. PCR products were then submitted for sequencing (Macrogen Inc, 10F, 254 Beotkkot-ro, Geumcheon-qu, Seoul, Republic of Korea). Sequences derived were submitted to GenBank.

Phylogenetic Analysis

Sequences for the 4b core protein gene derived from one *O. angolensis* (OA4) and one *O. crassirostris* (OC1) bird from Trinidad (O13_TT_2017 and A15_TT_2016, respectively), along with selected 4b core protein gene sequences from GenBank, were aligned using the ClustalW alignment tool within Geneious version 10.0.9 (Biomatters Ltd, Level 2, 18 Shortland Street, Auckland, 1010, New Zealand) and then trimmed (at the 5' and 3' ends) to a common length of 471 nucleotides. The final data set was comprised of 144 sequences from 19 countries isolated from 1980 to 2017. The best-fit evolutionary model for subsequent analyses¹³ was selected using jModelTest, which compares 52 models of nucleotide substitution using different model selection strat-

egies (hierarchical and dynamic likelihood ratio tests, Akaike and Bayesian information criteria, and a decision theory method).⁴ A maximum likelihood phylogeny was inferred under the selected model with 500 bootstraps performed using the PhyML method in Geneious version 10.0.9.⁶

RESULTS

All of the *O. angolensis* examined were in poor body condition with minimal subcutaneous and abdominal fat stores and markedly atrophic pectoral musculature. Multiple, variably coalescing, small, 2–4-mm-diameter, raised, proliferative, dark brown, firm, nodular foci were present in the tarsal area of the leg (OA1 and OA2) (Fig. 1), on the commissures of the beak (OA1–OA3), and on one or both eyelids, covering the eye (OA1–OA4). The lesions on OA2 and OA3 were variably ulcerated and hemorrhagic, being most marked in OA2. The crop and intestines of the birds were empty.

All five of the *S. intermedia* were in poor body condition with minimal subcutaneous and abdominal fat stores and markedly atrophic pectoral musculature. There were single to multiple, coalescing, raised, dark brown, firm nodules, 2–5 mm in diameter, with variable surface ulceration, hemorrhage, and scab formation on one or both eyelids (SP1–SP5), covering the eyes and impairing vision (SP3 and SP5).

The *O. crassirostris* (OC1) was in very poor body condition with a prominent keel bone, bilateral pectoral muscle atrophy, and multifocal, variably sized (1–2 mm), dark brown-gray, proliferative, friable masses around the nares.

All the gross proliferative, nodular lesions sampled had similar histological characteristics with variable ulceration of the epidermis, and the dermis was infiltrated with heterophils, macrophages, lymphocytes, and plasma cells. At the ulcerated borders, there was moderate epithelial hyperplasia with ballooning degeneration, and multiple cells contained large (12–16 μm) rounded eosinophilic cytoplasmic inclusions (Bollinger bodies) (Fig. 2). In one *O. angolensis* bird (OA1), necrosis and secondary infection extended into the underlying bone at the tarsus.

On electron microscopy, the cytoplasmic inclusions from one *O. angolensis* (OA3) contained large numbers of brick-shaped, enveloped, viral particles ranging from 297×107 nm to 346×196 nm (Fig. 3), which had a nucleocapsid and an envelope, consistent with avipoxviruses.^{1,16} A

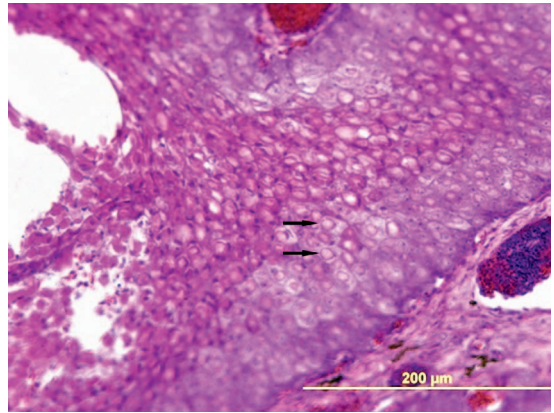


Figure 2. Poxviral inclusions (Bollinger bodies indicated by arrows) in a histologic section of the skin around the eyelid of *Oryzoborus angolensis*. Hematoxylin and eosin. $\times 40$.

large number of these viral particles were closely adhered to the viroplasm.

The 4b core-specific PCR confirmed the presence of avipoxvirus in all three species, and phylogenetic analysis of sequences derived from one *O. angolensis* (OA4) and one *O. crassirostris* (OC1) specimens (O13_TT_2017 [accession no. MG189368] and A15_TT_2016 [accession no. MG189367], respectively) showed that they were identical and belonged to the clade of canarypox-like viruses containing the majority of sequences

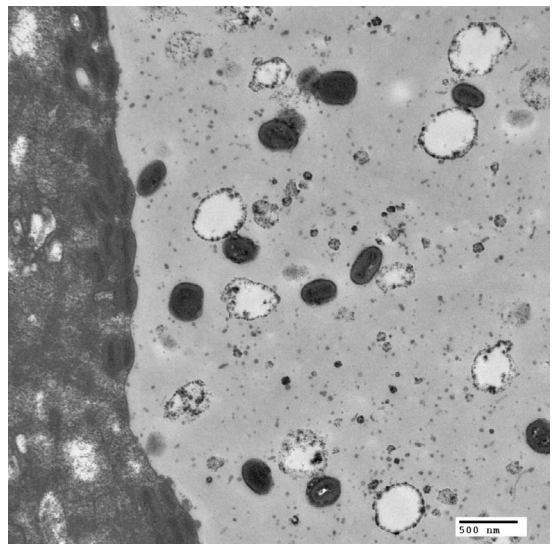


Figure 3. Transmission electron micrograph of poxviral particles (arrows) budding off the viroplasm (left) into the cytoplasm (right) in epithelium of the skin around the eyelid of *Oryzoborus angolensis*. $\times 10,000$.

from the Americas. They were most closely related to a 2012 sequence from a Brazilian penguin (*Spheniscus magellanicus*) sharing 96.2% and 98.1% identity at a nucleotide and amino acid level, respectively (Fig. 4).

DISCUSSION

In all three finch species, the gross lesions were typical of the cutaneous form of the disease caused by avian poxvirus infection, with multiple 1–5-mm-diameter nodules on nonfeathered areas. The eyelid lesions would have led to vision impairment, and the tarsal lesions would have compromised mobility, both resulting in a reduced ability to feed, even within the confines of a cage. The presence of Bollinger bodies, epithelial hyperplasia, hypertrophy, and ballooning degeneration histologically confirmed poxviral infection in all 10 finches.^{11,12,19} Electron microscopy demonstrated poxviral particles in one *O. angolensis* (OA3), with virions “budding” off the inclusion membrane, indicating a stage of viral production where the viruses are thought to acquire a second outer coat.³ Poxviral DNA was also detected by PCR in the three affected finch species.

Sequencing and phylogenetic analysis of the virus from one *O. crassirostris* and one *O. angolensis* specimen confirmed the presence of a canarypox-like virus that was distinct from but most closely related to a Brazilian penguinpox virus, reflecting a likely South American origin of the birds. It is widely known and law enforcement authorities in Trinidad would corroborate that most of the illegally imported birds originate from Venezuela, which is only approximately 10 km away. Venezuela shares borders with Brazil; however, the exact origin of the birds within Venezuela is unknown. Wildlife authorities have also indicated that a few birds may originate from Guyana and Suriname, which are also close to Venezuela. There is no existing knowledge of birds originating from Brazil.

The detection of this previously unreported virus, which was associated with clinical disease in illegally imported finches, suggests that the current practice of releasing illegally imported birds into the wild in Trinidad may have resulted in the spread this virus to native bird species. There are also concerns related to the displacement of native birds by the larger numbers of imported birds. The native Trinidad birds are typically larger and usually have a louder song than the South American birds, which may aid their continued existence. However, interbreeding

of the Trinidad and South American birds could lead to a loss of these native phenotypes, which, for birds which are prized for their song, would be devastating.

The poxvirus detected in this study could have originated in any of the three species of finch and then spread to the others, as the three species are often kept in close proximity in the same overcrowded cages for transport from the South American mainland to Trinidad. It has been proposed that all three species belong to the same *Sporophila* genus, which could also account for interspecies transmission in these birds.¹⁰ To the authors' knowledge, there have been no previous reports of poxvirus infection in these three finch species in South America. While age- and species-specific resistance is known to occur, both stresses associated with transit for smuggled birds and habitat displacement for native birds are likely to increase susceptibility of the birds to the virus.¹² Of the 10 birds examined, eight were juvenile, which is in accordance with the expectation of a more severe manifestation of disease in juvenile birds.¹² Trinidad has a tropical climate with year-round mosquito activity, making mosquito transmission a likely factor in disease spread in the country. Furthermore, the virus may be shed for up to 13 mo after clinical disease;¹⁶ thus, the release of sick birds, birds with subclinical or unapparent infection, or even recovered birds into the wild could lead to its onward spread to native susceptible species.

The results from this study emphasize the need to control and prevent the illegal movement of wild birds from the South American mainland to the island of Trinidad in order to stop the spread of potentially devastating diseases like avian pox. Determining the genetic structure of these important songbirds in the region would assist with tracing the origin of the illegally imported birds. These results also point toward a need to review current practices on the treatment of illegally imported birds seized by state authorities to ensure that birds are not released into the wild to the detriment of native bird populations. Development of an appropriate quarantine and testing center for these birds prior to release or on-site euthanasia is a more appropriate action with respect to disease prevention and control, which are currently a problem. Enforcement of animal welfare laws to prevent overcrowding and other captive management issues is currently a problem with inadequate staffing for the wildlife law enforcement section, coupled with the likelihood that wildlife smuggling from the mainland is

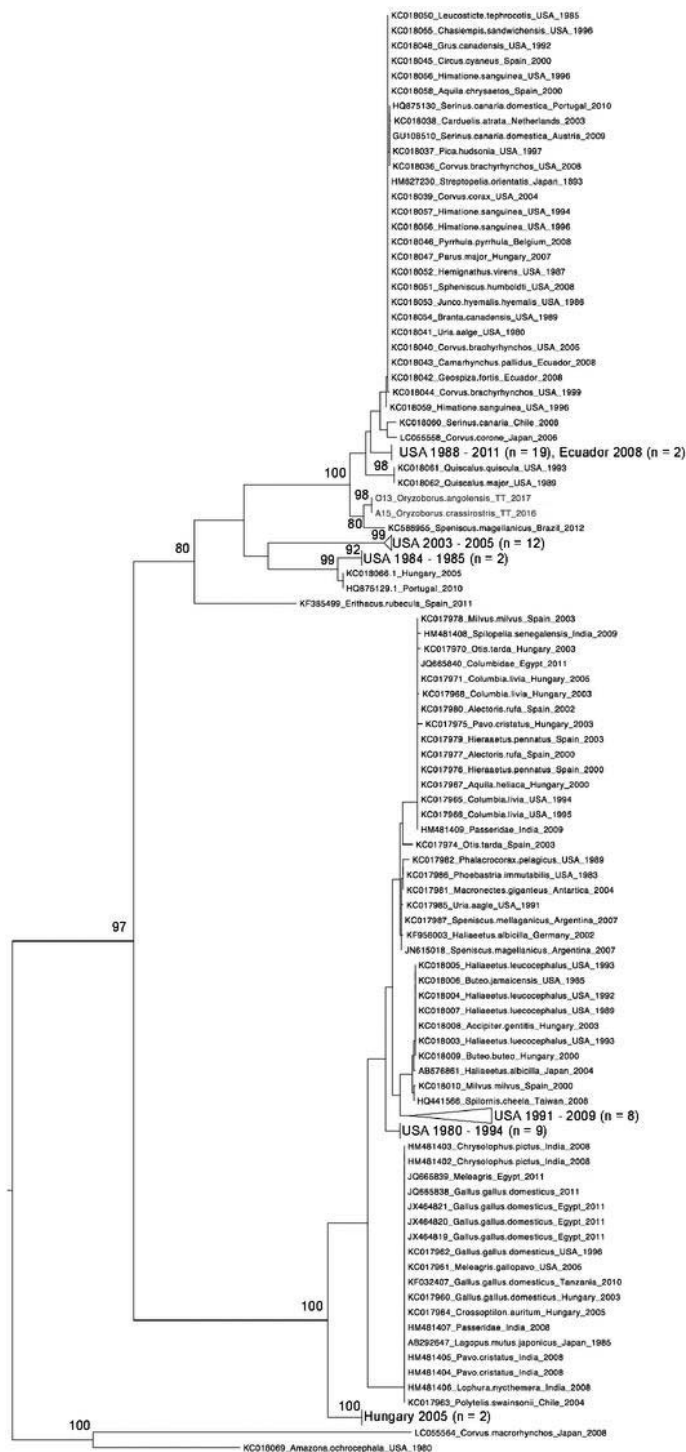


Figure 4. Maximum likelihood phylogeny of 144 poxvirus 4b core coding sequences. Branch tips are labeled with each sequence's GenBank accession number, country, and year of isolation. Sequences from Trinidad and Tobago isolates are highlighted in blue. The country and year of isolation followed by the number of sequences (n) comprising collapsed clades (in brackets) are shown next to the relevant clade. Bootstrap values over 80% are indicated at the respective nodes.

likely to be associated with more nefarious smuggling activities. Captive breeding of *O. angolensis*, the most popular of the three species in Trinidad, is an industry in Brazil, and development of this industry in Trinidad is a sustainable option for conservation.

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LITERATURE CITED

1. Bolte AL, Meurer J, Kaleta EF. Avian host spectrum of avipoxviruses. *Avian Pathol.* 1999;28(5):415–432.
2. Calabuig P, Casal AB, Camacho M, Orós J. Poxvirus infection in stone curlews in the Canary Islands. *Vet Rec.* 2011;168(6):168.
3. Cheville NF. Cytopathologic changes in fowlpox (turkey origin) inclusion body formation. *Am J Pathol.* 1966;49(4):723–737.
4. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods.* 2012;9(8):772–772.
5. Ffrench R. A guide to the birds of Trinidad and Tobago. 2nd ed. London (United Kingdom): Christopher Helm; 2000. p. 386–392.
6. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol.* 2003;52(5):696–704.
7. Gyuranecz M, Foster JT, Dán A, Ip HS, Egstad KF, Parker PG, Higashiguchi JM, Skinner MA, Höfle U, Kreizinger Z, Dorrestein GM, Solt S, Sós E, Kim YJ, Uhart M, Pereda A, González-Hein G, Hidalgo H, Blanco JM, Erdélyi K. Worldwide phylogenetic relationship of avian poxviruses. *J Virol.* 2013;87(9):4938–4951.
8. Kirmse P, Loftin H. Avian pox in migrant and native birds in Panama. *Bull Wildl Dis Assoc.* 1969;5(2):103–107.
9. Lee LH, Lee KH. Application of the polymerase chain reaction for the diagnosis of fowl poxvirus infection. *J Virol Meth.* 1997;63(1–2):113–119.
10. Mason NA, Burns KJ. Molecular phylogenetics of the neotropical seedeaters and seed-finches (*Sporophila*, *Oryzoborus*, *Dolospingus*). *Ornitol Neotrop.* 2013;24:139–155.
11. Schoemaker NJ, Dorrestein GM, Lumeij JT. An aviapoxvirus infection in a goshawk (*Accipiter gentiles*). *Avian Pathol.* 1998;27(1):103–106.
12. Smits JE, Tella JL, Carrete D, Serrano D, López G. An epizootic of avian pox in endemic Short-toed Larks (*Calandrella rufescens*) and Berthelot's Pipits (*Anthus berthelotti*) in the Canary Islands, Spain. *Vet Pathol.* 2005;42(1):59–65.
13. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol.* 1993;10(3):512–526.
14. Tangredi BP. Avian pox in a mourning dove. *Vet Med Small Anim Clin.* 1974;69(6):700–701.
15. Tikasingh ES, Worth CB, Spence L, Aitken THG. Avian pox in birds from Trinidad. *J Wildl Dis.* 1982;18:133–139.
16. Tripathy DN, Reed WM. Pox. In: Saif YM, Glisson JR, Fadly AM, McDougald LR, Swayne DE (eds.). *Diseases of poultry*, 11th ed. Ames (IA): Iowa State University Press; 2003. p. 253–269.
17. US Department of the Interior and US Geological Survey. *Field manual of wildlife diseases: general field procedures and diseases of birds.* Washington (DC): US Geological Survey; 1999. p. 163–170.
18. Van Riper C, Forrester DJ. Avian Pox. In: Thomas NJ, Hunter DB, Atkinson CT (eds.). *Infectious diseases of wild birds.* Ames (IA): Blackwell Publishing Professional; 2007. p. 131–176.
19. Van Riper C, Van Riper SG, Hansen WR. Epizootiology and effect of avian pox on Hawaiian forest birds. *Auk.* 2002;119(4):929–942.

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