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WHOLE GENOME SEQUENCING OF AN AVIPOXVIRUS ASSOCIATED WITH INFECTIONS IN A GROUP OF AVIARY-HOUSED SNOW BUNTINGS (*PLECTROPHENAX NIVALIS*)

Rozenn Le Net, DV, Chantale Provost, PhD, Christian Lalonde, MSc, Lyette Régimbald, MSc, François Vézina, PhD, Carl A. Gagnon, DMV, PhD, and Stéphane Lair, DVM, DVSc, Dipl ACZM

Abstract: Avipoxvirus infections have been reported in both free-ranging and domestic birds worldwide. Fowlpox and canarypox viruses belong to the genus *Avipoxvirus* among the virus family *Poxviridae*. They cause cutaneous lesions with proliferative growths on the unfeathered parts of the skin and/or diphtheritic lesions generally associated with necrosis in the upper respiratory and digestive tracts. In this study, a poxvirus has been identified in wild-caught snow buntings (*Plectrophenax nivalis*) housed in an outdoor aviary in the region of Rimouski, Quebec. During the falls and winters of 2015 and 2016, eight snow buntings affected by this infection were examined. Macroscopic and microscopic lesions observed were characteristic of an avipoxvirus infection. Electron microscopy imaging of an ultrathin section of the histopathological lesions of two birds confirmed the presence of the poxvirus. Afterward, the presence of the poxvirus was confirmed in three birds by a specific polymerase chain reaction assay that amplified a segment of the gene encoding the fowlpox virus 4b core protein. A 576-nucleotide amplicon was obtained from one of them and sequenced. The analyses revealed a 99% homology to other previously described avipoxviruses. Using high-throughput sequencing, almost the entire viral genome of this avipoxvirus was revealed and found to possess a 359,853-nucleotide sequence in length. Bioinformatic analyses revealed that the virus was genetically related to canarypox virus. To our knowledge, this is the first confirmed case and full description of a poxviral infection in this species. This episode suggests a high susceptibility of this northern species of passerine to avipoxviruses circulating in southeastern Canada during the summer months. Even if the source of the viral infections remains undetermined, transmission by local biological vectors is suspected. Management of poxviral infections in snow buntings housed outdoors in southeastern Canada could rely on the control of biting insects.

Key words: Avipoxvirus, canarypox, pathology, *Plectrophenax nivalis*, pox, snow bunting, whole genome sequencing.

INTRODUCTION

Avipoxviruses have a worldwide distribution and have been shown to naturally infect more than 232 species of birds, belonging to 23 orders,[19] including the order Passeriformes.[29] Avipoxviruses belong to the *Poxviridae* family in the *Chordopoxvirinae* subfamily and are made up of three major clades, with most of characterized isolates classified in clade A (of which fowlpox virus is the most common species) or clade B (of which canarypox virus is the most common specie).[8] Sporadic cases of poxviral infections, which are characterized mainly by the presence of wart-like masses on unfeathered areas,[2] have been reported in various groups of free-ranging birds, including corvids,[20] columbidae,[28] passerines,[14] hummingbirds,[7] raptors,[27] and galliforms.[6] Epizootics of infection by viruses of the *Poxviridae* family have also been reported in colonial nestling birds, such as lesser flamingos (*Phoenicopterus minor*),[33] southern giant petrel (*Macronectes giganteus*),[26] and Laysan albatross (*Phoebastria immutabilis*).[32] Even if some studies did not show any significant impact of this viral disease on fledgling success,[26,32] infections by poxviruses have been proposed to decrease fitness in Galapagos finches,[12] lesser short-toed lark (*Calandrella rufescens*),[25] and great tits (*Parus major*).[14] Introduced poxviruses are also believed to be a contributing factor in the decline of some populations of endemic birds from Hawaii.[30]

Reports of poxvirus infections in birds under human care are not as common as in free-ranging

From Centre québécois sur la santé des animaux sauvages/Canadian Wildlife Health Cooperative, Faculté de médecine vétérinaire, Université de Montréal, 3200 rue Sicotte, St. Hyacinthe, Québec J2S 2M2, Canada (Le Net and Lair); Centre de recherche en Infectiologie Porcine et Avicole (CRIPA), Laboratoire de diagnostic virologique vétérinaire et de diagnostic moléculaire, Faculté de médecine vétérinaire, Université de Montréal, 3200 rue Sicotte, Saint-Hyacinthe, Québec J2S 2M2, Canada (Provost, Lalonde, and Gagnon); Département de biologie, chimie et géographie, Université du Québec à Rimouski, 300 allée des Ursulines, Rimouski, Québec G5L 3A1, Canada (Régimbald and Vézina). Present address (Le Net): Pôle Expertise Vétérinaire et Agronomique Animaux Sauvages, VetAgro Sup, 1 avenue Bourgelat, 69280 Marcy l'Etoile, France. Correspondence should be directed to Dr. Lair (stephane.lair@umontreal.ca) and Dr. Gagnon (carl.a.gagnon@umontreal.ca).

birds. Poxviral infections have been documented in different species of flamingos housed in zoological institutions.[1,9,21] This lower occurrence in captive birds compared to free-ranging ones is likely due to a lower exposure to blood-sucking or biting arthropods, which are believed to be the main route of transmission of poxviruses.[2] This disease has been well documented in a captive breeding program of houbara bustards (*Chlamydotis* spp.), in which it seems to have a low impact on breeding success.[16]

Snow buntings, small passerine birds, have a very wide circumpolar distribution range.[18] During winter, this species can be found throughout the northern half of the United States and southern Canada. In the spring, the North American population migrates northward to breed along the coasts of Greenland, in the Canadian Arctic, and in Alaska. In the past 40 years, snow buntings have experienced a population decline of 64% throughout North America or at least throughout regions surveyed.[3] Even if this species has been the subject of numerous biological and ecological studies, little is known on its diseases and causes of death.

Over the past few years, studies on snow buntings' metabolism have been conducted at the Université du Québec à Rimouski. During the falls and winters of 2015 and 2016, outbreaks of poxviral infections have occurred in snow buntings housed in an outdoor research facility located on university grounds. We report here the clinical presentation, pathological findings, and genomic sequence and phylogenetic classification of the avipoxvirus associated with these cases. We also discuss the potential source of infection of these wild-caught aviary-housed birds in the context of the first full description of such a viral infection in this species.

MATERIALS AND METHODS

The affected birds belonged to a group of about 30 mainly male research birds used in physiology studies at the Université du Québec à Rimouski. These studies were approved by the Institutional Animal Care and Use Committee of the Université du Québec à Rimouski, which operates under the auspices of the Canadian Council on Animal Care. These wild birds were captured from January to March in the vicinity of Le Bic, Quebec, Canada (48.3756°N, 68.6952°W). Two of the affected birds were captured in 2013, one in 2014, three in 2015, and two in 2016. No abnormality was noted on capture after a complete physical examination. The birds were first maintained in quarantine in an indoor cage for 1 wk and then separated by sex in two outdoor aviaries on university ground (48.4526°N, 68.5121°W). These aviaries were part of a research building composed of four outdoor aviaries, including two housing a group of black-capped chickadees (*Poecile atricapillus*). Each aviary is separated by a single wire grid, enabling direct contact between male and female snow buntings and between female snow buntings and black-capped chickadees. Double fencing around the exterior of the aviaries prevents direct contact with free-ranging birds. The research facility also housed zebra finches (*Taeniopygia guttata*), pine siskins (*Spinus pinus*), and black-capped chickadees in indoor rooms in the research building. No contact between the indoor birds and the snow buntings was reported, and all the equipment used was dedicated to each species exclusively. The diet of the snow buntings consisted mainly of seeds, namely, cut corn, wheat, red milo, white millet, red millet, black oil sunflower (All Season Feather Treats, Armstrong Milling Company, Ltd, Hagersville N0A 1H0, ON, Canada), and dry bird food (Small Bird Maintenance Mini Diet, Mazuri, PMI Nutrition International, LLC, St. Louis, MO 63166, USA). Fresh water, supplemented with Electrolyte Plus (Vetoquinol North America, Inc, Lavaltrie, Quebec J5T 3S5, QC, Canada) or Poly-tonine A® Complex (Vetoquinol North America) was provided when outdoor temperatures were above freezing. During the wintertime, the birds had access to unpacked snow.

All affected birds presented clinical signs characterized by the presence of a single or multiple small dermal, usually proliferative lesions on the legs, wings, and face. Symptomatic birds were isolated in an indoor birdcage. Several treatments were attempted without success, including diluted chlorhexidine bath, anti-inflammatory topical ointment (Taro-Mupirocin 2%, Taro Pharmaceuticals, Inc, Brampton L6T 1C1, ON, Canada), Derma Gel® (Maximilian Zenho & Co, Inc, Ocala, FL 34482, USA), topical treatment with a dexamethasone and tobramycin ophthalmic ointment (Tobradex®, Novartis Pharmaceuticals 105 Canada, Inc, Dorval H9S 1A9, QC, Canada), and oral tetracycline/neomycin (0.9 g in 1 L of water for 7–14 days; Neo-Chlor manufactured by Vetoquinol North-America). One of the affected birds (#8) had been vaccinated against fowlpox virus and avian encephalomyelitis (AE-Poxine, Zoetis Canada, Inc, Kirkland H9H 4M7, QC, Canada) in March 2016, 6 mo prior to

the onset of the clinical signs. Four birds died naturally, whereas three were euthanized. Bird #6 was amputated due to the presence of a leg fracture. A mass was noted on the intertarsal joint, and the leg was submitted for histopathology. None of the birds from the other species kept in this facility exhibited lesions suggestive of an avipoxvirus infection.

Following the documentation of the first case, perches and equipment were disinfected once a month with Virkon 1% (Vetoquinol N.-A Inc, Lavaltrie J5T 3S5, QC Canada) as per the recommendation of the company.

Standard necropsies were realized on frozen thawed carcasses. A body condition score from 1 to 5 was attributed according to the size of keel musculature; a score of 1 was attributed to an emaciated bird, whereas an obese bird was given a score of 5.[31] Tissue samples were fixed in 10% buffered formalin; embedded in paraffin; sectioned at 3 μm; stained with hematoxylin, phloxine, and saffron; and examined by light microscopy. Additional samples were kept frozen (−20°C).

Paraffin-embedded damaged skin fragments of two birds were processed for transmission electron microscopy (TEM). Paraffin bloc sections were cut with a scalpel blade, and tissues were deparaffined in xylene and rehydrated with decreasing concentrations of ethanol. Then coloration and fixation of the deparaffined tissues were performed in OsO_4 2%. Tissues were dehydrated again with increasing concentrations of ethanol. Embedding of the samples was done using acetonitrile and Embed812 resin. Ultrathin sectioning was performed with a PC-PT PowerTome ultramicrotome (RMC-Boeckeler Instruments, Tucson, AZ 85714, USA) using glass knifes made with a LKB 7800 KnifeMaker (LKB-Produkter AB, S-161 25 Bromma, Sweden). Ultrathin sections, about 60–70 nm thick, were laid down on copper grids 400 mesh negatively charged with a carbon evaporator Q150T (Quorum Technologies, Ltd, Laughton, East Sussex BN8 6BN, United Kingdom). Postcoloration was performed using uranyl acetate and lead citrate. Visualization and pictures of skin lesions from the two birds were done on an HT7700 transmission electronic microscope (Hitachi High Technologies Canada, Inc, Toronto M9W 6A4, ON, Canada) at 60 kV.

Prior to molecular biology testing, damaged tissue samples from four birds were unfrozen, whereas for the four others, formalin-fixed and paraffin-embedded tissues were dewaxed and rehydrated. A polymerase chain reaction (PCR) assay that amplified a segment of the gene encoding for the fowlpox 4b core protein[17] was performed on damaged tissue from the eight birds. DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Inc, Toronto M5J 2T3, ON, Canada). PCR was performed with primers P1 forward, CAGCAGGTGCTAAACAACAA, and P2 reverse, CGGTAGCTTAACGCCGAATA. The 50-μl PCR mix contained 10× DreamTaq Buffer and includes 20 mM $MgCl_2$ (Thermo Fisher Scientific, Ottawa K2E 7L6, ON, Canada), 0.25 mM of each dNTP (New Englands Biolabs, Whitby L1N 9T7, ON, Canada), 1 mM of each primer (Integrated DNA Technologies, Inc, Skokie, IL 60076, USA), 1.25 U of DreamTaq DNA Polymerase (5 U/μl) (Thermo Fisher Scientific), and 5 μl of diluted/undiluted DNA or control. PCR cycles were done as follows: first, an initial denaturation step at 95°C for 3 min; then 40 cycles at 95°C for 30 sec, 50°C for 30 sec, and 72°C for 1 min; and a final elongation step at 72°C for 7 min. PCR amplicons were collected from a 1% agarose gel, purified with a QIAquick Gel Extraction Kit (Qiagen), and sequenced by the Sanger sequencing services at the Faculté de médecine vétérinaire, Université de Montréal (Canada).

To obtain the full-length genome of the avipoxvirus, high-throughput sequencing was performed on one of the skin fragments. DNA was extracted as mentioned before. Library was carried out using Nextera XT DNA library preparation kit (Illumina Canada Inc, Vancouver V8Z 7X8, BC, Canada) following the manufacturer's instructions. Briefly, 1 ng of DNA was tagmented with 10 μl Tagment DNA Buffer and 5 μl Amplicon Tagment Mix at 55°C for 5 min, followed by hold at 10°C using a Thermocycler TProfessional Basic 96 (Biometra GmbH, 37079 Göttingen, Germany). The transposome was stopped with 5 μl of Neutralize Tagment Buffer. Library was amplified using index adapter and Nextera PCR Master Mix following those PCR steps: 72°C for 3 min, 95°C for 30 sec, and then 12 cycles of 95°C for 10 sec, 55°C for 30 sec, 72°C for 30 sec, and a final elongation step at 72°C for 5 min and hold at 10°C. Library was cleaned with Axygen™ AxyPrep Mag™ PCR Clean-up Kits (Thermo Fisher Scientific). Library's quality and size was performed with a Highly Sensitive DNA chip on an Agilent 2100 Bioanalyzer (Agilent Technologies Canada, Inc, Mississauga L5N 5M4, ON, Canada). Library was normalized and converted to single-stranded DNA with Library Normalization Beads 1 (LNB1) beads and 0.1 N NaOH. Library

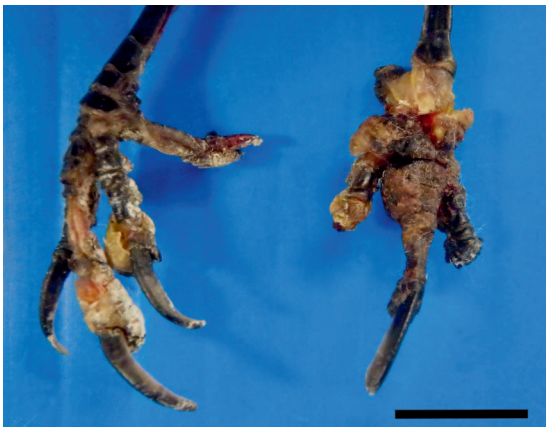

Figure 1. Legs of an adult male wild-caught aviary-housed snow bunting (*Plectrophenax nivalis*; bird #1) showing marked proliferative ulcerative dermatitis with crusting. Note the missing digits. Bar = 1 cm.

was denatured at 98°C for 2 min, and 1% PhiX was used as an internal control for the v3 600 cartridge (Illumina Canada Inc). Sequencing was done on MiSeq (Illumina Canada Inc), in two different runs. Sequences analysis was performed with CLC workbench (Qiagen, Inc) using *de novo* and resequencing work flows. The total reads (5,347,284) from both runs were pooled and mapped against various reference poxvirus genomes. The 0.38% (20,393) reads that mapped to poxvirus were then used to assemble contigs *de novo*. Contigs were mapped against the same references previously used, and the poxvirus genome in which most contigs mapped was subsequently used as the reference (canarypox; GenBank accession number NC005309) for further analyses. The poxvirus reads were then mapped to reference NC005309 alongside the contigs obtained previously, and the full genome was obtained. Annotations were added to the genome based on the reference canarypox genome (NC005309).

Two maximum likelihood phylogeny trees based on a 428-nucleotide fragment of the core 4b gene were made using the CLC Genomics Workbench software on default settings, one showing various chordopoxviruses and one showing a range of avipoxviruses. The trees were made using the Juke–Cantor substitution model (Kimura 80, generalized time reversible, and HKY85 models offered the same layout, accuracy, and resolution), with a bootstrap setting of 1000 and shown as unrooted. Twenty-seven partial 4b core protein sequences were selected and obtained from GenBank and 27 based on host species to build diverse but concise and accurate trees.

RESULTS

Gross pathology

All birds examined were adults; five were males, and three were females. Four birds were considered to be in good to very good body condition, one was thin, two were emaciated, and one was not assessed. Every bird displayed proliferative cutaneous lesions characterized by a thickening of the skin or oral mucosa forming occasionally ulcerated masses covered with crust (Fig. 1). These masses were located on toes, intertarsal articulations, eyelids, wrists, elbows, and oral mucosa. Swelling of the intertarsal joint was observed in one of the birds, whereas complete ankylosis of the elbow or interphalangeal joint was present in three other birds. One bird showed a 2 × 1-cm, well-defined cloacal mass that was adhered to the cloacal wall. One bird had several missing digits.

Histopathology

Histopathologic examination of the cutaneous lesions showed lesions characteristic of avipoxvirus infection: marked acanthosis with hypertrophy of epidermal cells, often showing ballooning degeneration, marked hyperkeratosis with accumulation of debris, blood and bacteria at the surface of the epidermis, the presence of numerous large eosinophilic intracytoplasmic inclusion bodies (Bollinger bodies) filling a large proportion of the cytoplasm of epidermal cells, areas of epidermal necrosis associated with infiltrations of degenerated inflammatory cells, and mononuclear inflammatory infiltrations of the dermis (Fig. 2). Cellulitis, osteomyelitis, and/or arthritis with intralesional bacteria were observed in four cases (#1, #4, #6, and #7). The cloacal mass observed in one of the birds (#8) was formed by a large granuloma between the coelomic and cloacal walls, centered on Gram-positive cocci and associated with a markedly thickened hyperplastic cloacal mucosa in which numerous intracytoplasmic inclusion bodies could be observed.

Based on the characteristic findings, a tentative diagnosis of avipoxvirus infection was made for all birds examined. Details on clinical presentation for each case are provided in Table 1.

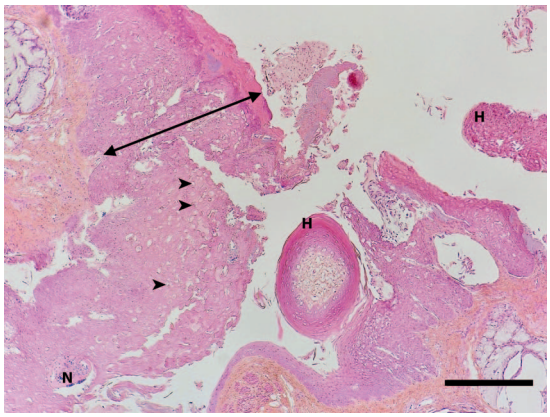

Figure 2. Section from the oral mucosa of an adult female wild-caught aviary-housed snow bunting (*Plectrophenax nivalis*; bird #5) showing areas of acanthosis characterized by a marked hyperplasia of the epithelium (double-headed arrow) with ballooning degeneration and Bollinger bodies (arrowhead), hyperkeratosis (H), and areas of epidermal necrosis (N). Hematoxylin, phloxine, saffron stain. Bar = 500 μm.

Electron microscopy

Electron microscopic examination of fragments skin and mucosa from two birds revealed that many large cytoplasmic inclusion bodies within epidermal cells contained numerous viral particles (Fig. 3). The virus morphology was characteristic, with an overall ovoid shape with lateral bodies present in the concave region between the core wall and a membrane, which possesses an approximate size of 200 by 400 nm, confirming that those viruses belonged to the *Poxviridae* family.

Molecular detection and characterization of the virus

PCR assays were positive for three birds out of the eight tested, including two birds for which unfrozen tissue samples were tested and one bird for which a formalin-fixed tissue sample was tested. One sample yielded an amplification band of about 580 bp, from which we recovered a 576-bp sequence. The analyses revealed a 99% identity to other previously described avipoxviruses (GenBank accession numbers AY453173 and AY530309, among others). The full-length genome of 359,853 bp with an average coverage of 17.65 was obtained using high-throughput sequencing with the MiSeq sequencing platform (Illumina Canada Inc). The open reading frames (ORFs) illustrated in Figure 4 are described in detail within Supplemental Table 1. A total of 328 putative and confirmed ORFs have been identified by comparing our sequence to the previously published *Canarypox virus* genome (GenBank accession number NC005309).

Two phylogenetic trees were also done comparing a 428-bp fragment of the core 4b protein from the snow bunting poxvirus sequence to other chordopoxviruses (Fig. 5a) and avipoxviruses (Fig. 5b). Subsequent comparison of the partial 4b core gene sequence showed that the snow bunting avipoxvirus sequence is very close to reported *Canarypox virus* species and is a member of clade B avipoxvirus, as illustrated in Figure 5b. In fact, the nucleotide identity between the snow bunting avipoxvirus with other clade B avipoxvirus was between 84.35% and 99.77% and was only 75%–76.4% when compared to clade A avipoxviruses.

Table 1. Clinical information on the cases of avipoxvirus infection in wild-caught aviary-housed snow buntings (*Plectrophenax nivalis*) documented in a research facility of the Department of Biology, Chemistry, and Geography of the Université du Québec à Rimouski, Rimouski, Quebec (Canada).

Case no.	Date captured	Date of death (outcome[a])	Sex	BCS[b]	Affected sites	Electron microscopy	PCR result[c]
1	Feb 2013	Oct 2015 (E)	Male	2	Toes	Not tested	Negative*
2	Jan 2015	Oct 2015 (N)	Female	3	Eyelids and oral mucosa	Not tested	Negative*
3	Jan 2015	Nov 2015 (N)	Female	4.5	Eyelids	Not tested	Positive
4	Feb 2013	Oct 2015 (E)	Male	1.5	Elbow	Not tested	Negative
5	Jan 2015	Nov 2015 (N)	Female	4.5	Toe and oral mucosa	Positive	Positive
6	Jan 2016	May 2016 (A)	Female	Unk.[d]	Leg	Positive	Negative
7	Unk.	Sept 2016 (N)	Male	1	Foot	Not tested	Negative*
8	Mar 2016	Oct 2016 (E)	Male	4	Eyelid, toes, wrist, cloaca	Not tested	Positive*

[a] E indicates euthanasia; N, natural death; A, alive (only the leg was submitted).
[b] BCS indicates body condition score: 1, emaciated; 2, thin; 3, suboptimal; 4, optimal; 5, overweight.
[c] PCR indicates polymerase chain reaction. An asterisk indicates that the DNA was obtained from deparaffinized tissues; otherwise, it was obtained from frozen tissues.
[d] Unk. indicates unknown.

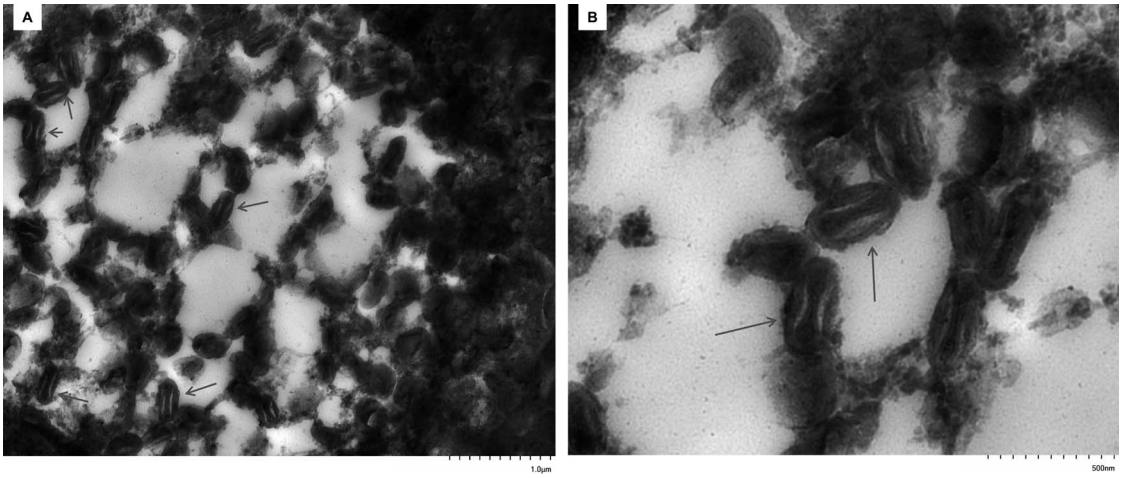

Figure 3. Transmission electron microscopy images of ultrathin sections of the skin lesion of the right leg of an affected snow bunting (*Plectrophenax nivalis*). Visualization was done on a Hitachi HT7700 transmission electron microscope. Poxviruses are pointed with arrows. A) Magnification of ×12,000 at 60 kV. Scale bar = 1 µm. B) Magnification of ×30,000 at 60 kV. Scale bar = 500 nm.

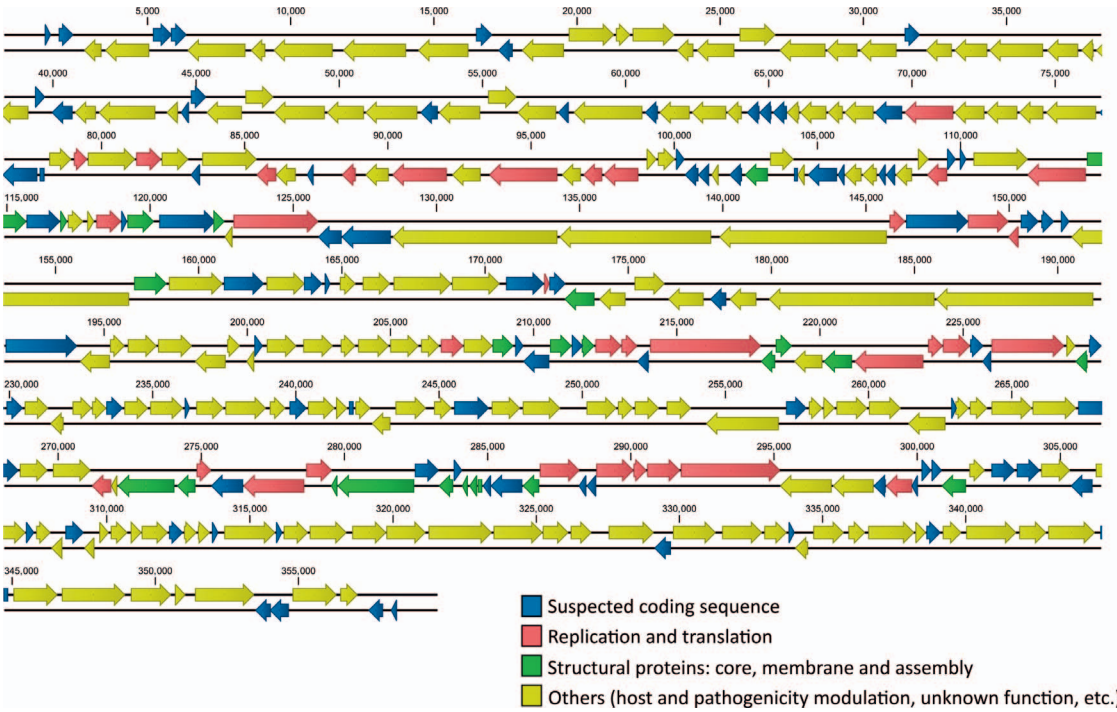

Figure 4. Schematic representation of known and putative open reading frames (ORFs) of the new snow bunting canarypox virus complete genome. The full-length genome of the snow bunting canarypox virus is 359,853 nucleotides in length (GenBank accession number MG760432). The arrows represent the localization of the ORFs within the viral genome, and their functions are indicated by a color code (see legend in the figure). The numbers on the top of the arrows represent the nucleotide positions. The name and nucleotide positions of each ORF can be found in Supplemental Table 1.

DISCUSSION

Even if the presence of a poxvirus was confirmed by either molecular diagnostic or electron macroscopy in only four of the cases, a diagnostic of poxviral infection was given to all eight birds due to the presence of highly characteristic histologic lesions. This is the first complete description of cases of poxvirus infection in snow buntings. Irons, in 1934, described the pathogenic effect of *Pigeonpox virus* in artificially infected snow buntings, but little information was given.[10] Clinical presentation and pathological findings were typical of a poxvirus infection.[2,19,22] Two of the three forms of infection,[22] the cutaneous (or dry) and the internal diphtheroid (or wet), were observed in the cases reported here. Cutaneous lesions, namely, wart-like masses, occasionally ulcerated and/or crusted, mostly on unfeathered areas, were characteristic of infection by an avipoxvirus.[2,19,22] As with any avipoxvirus, the wet form was observed less frequently than the cutaneous form;[19] two of the eight birds were showing lesions of the oral mucosa. Mortality rate with the cutaneous form of poxvirus infection is usually low.[22] Nonetheless, in the epornitic described here, all the birds that showed pox lesions at the facility either died naturally or were euthanized for welfare reasons. In two of the birds that died naturally, the severity of the lesions and the poor body condition of the birds suggest that dysphagia associated with the presence of pox lesions contributed to the mortality. Even if the cause of death remains uncertain in the two other birds that had mild focal lesions and were in very good body condition, it can be speculated that the secondary bacterial coinfections observed contributed to these fatalities.

TEM pictures clearly demonstrate the presence of poxvirus in two samples. However, discordance in the PCR results in one of the positive samples obtained by TEM was observed. This discrepancy could be explained by the difference in homology of the primers used. P1 primer has a 95% homology to our canarypox virus sequence, whereas P2 primer has only an 80% homology. Moreover, a divergence between negative PCR results and positive characteristic histologic lesions was also observed. It is well known that DNA extracted from deparaffinized tissue often leads to a lower DNA quality and yield,[24] and half of the samples tested by PCR were from deparaffinized tissues. Thus, the inconsistency in the results might come from the type of samples analyzed by PCR in combination with an 80% homology of one of the primers used, which could overall reduce significantly the sensitivity of the PCR assay.

Once sequenced, it appeared that the genome length and organization of the snow bunting avipoxvirus was very similar to that of canarypox viruses, therefore belonging to clade B of avipoxvirus. Typically, avipoxvirus is composed of three clades; clade A, of which fowlpox virus is the most well-known species; clade B, of which canarypox virus is the most well-known species; and clade C, which is made up mostly of psittacinepox virus.[7,11] The 4b segment sequenced clearly places the snow bunting's avipoxvirus in clade B. The snow bunting avipoxvirus sequenced in this study also shared 99% nucleotide identity over its whole genome with the reference canarypox virus (GenBank accession number NC005309), further confirming its phylogenomic identification.

Since 1992, a total of 29 free-ranging snow buntings has been examined by the diagnostic facilities of the Canadian Wildlife Health Cooperative. None of these birds presented with lesions suggestive of avipoxvirus infection (CWHC National Wildlife Health Database). Little is known about diseases and causes of death in snow buntings; hardly any publications have hitherto focused on diseases in snow buntings. Only one case report in the literature describes a diseased snow bunting, which probably died from salmonellosis.[4] A few publications have documented parasitic infections in snow buntings, without evidence of any pathogenic effect on birds. Nasal mites (*Ptylonyssus* genus) have been reported in Manitoba (Canada).[13] Dolnik *et al*. described for the first time the infection of juvenile snow buntings by an avian isosporan parasite, *Isospora plectrophenaxia*, again without evidence of any pathogenic effect on birds.[5] The lack of knowledge in diseases of snow buntings may stem from their small size, their use of rural habitat with low human population, and their northern distribution range resulting in challenges in getting access to carcasses. Consequently, because of this limited documentation of causes of death in this species, the presence (or absence) of this infection in the free-ranging snow bunting population remains undetermined.

Nevertheless, the occurrence of this episode in a captive setting within an unusual summer range for this species suggests that captivity have created artificial conditions leading to a poxviral infection in this group of birds. Even if these birds could have been asymptomatic carriers, the absence of lesions at capture suggests that this virus was acquired in the research facility. Obviously,

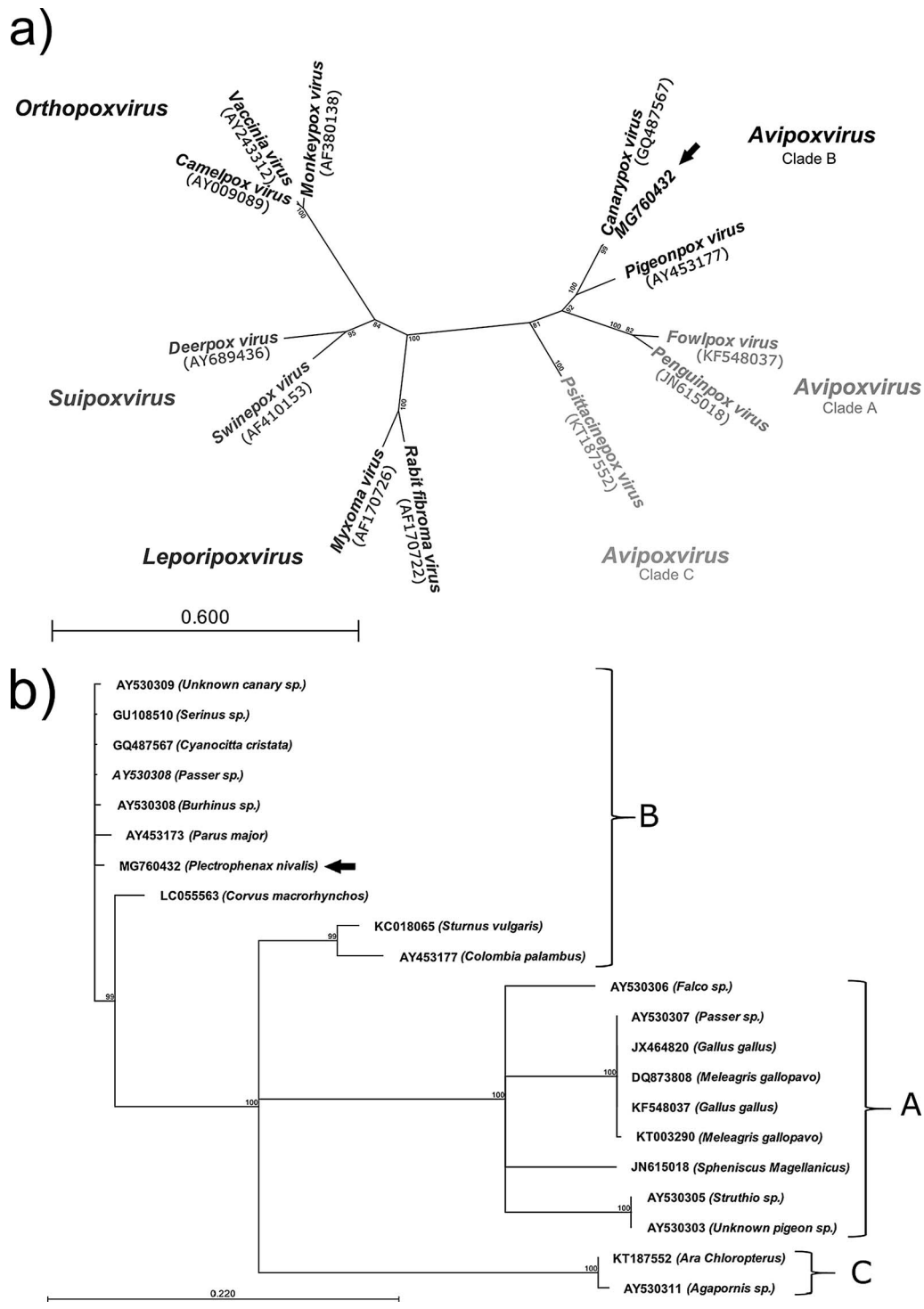

Figure 5. Phylogenetic analysis of the snow bunting avipoxvirus core 4b gene partial nucleotide sequences. Phylogenetic trees of a 428 nucleotides fragment of the core 4b gene comparing the snow bunting's avipoxvirus to a) various chordopoxviruses and b) various avipoxviruses. Virus strains are identified with their GenBank accession number with a) the species of the poxvirus and b) the host birds from which the viruses were isolated. The three main avipoxvirus clades are depicted; clade A (mainly fowlpox virus), clade B (mainly canarypox

being kept in southern Quebec during the warm months of the year, they were exposed to unusual vectors, including blood-sucking or biting arthropods, that could have facilitated transmission of a virus to which this species is naive.[2] Seven of the eight cases were documented in the fall. This seasonal pattern, following the summer season with a high abundance of mosquitos, is similar to what is observed in other bird species[22,29] and supports the hypothesis of an arthropod-borne transmission.[15] Other possible routes of transmission are direct contact, aerosols, fomites, and contaminated food and water.[22,29] Disinfection of perches and equipment did not appear to prevent the occurrence of this disease. This is not surprising since the efficacy of the disinfection of nonprotected porous material like wood is likely quite limited. Direct contact with free-ranging birds is unlikely due to the presence of double-wire mesh. Alternatively, transmission from direct contact with asymptomatic black-capped chickadees housed in the adjacent pen cannot be ruled out. However, the highest number of cases in male birds, which did not have direct contact with the chickadees, do not support this. In addition, poxviral infection has never been reported in black-capped chickadees, and the absence of poxviral disease in black-capped chickadees, kept in the same type of outside aviaries, whereas snow buntings showed severe lesions, leads us to believe that black-capped chickadees are somewhat resistant to this avipoxvirus. The absence of cases of poxviral infection in terrestrial Arctic bird species[29] may suggest that this disease is not present in this ecosystem.

The captive setting might have enhanced the transmission of the avipoxvirus by increasing bird-to-bird contact due to their confinement. As for most viral diseases, transmission of avipoxviruses is promoted when the host density increases.[29] Alternatively, poxvirus could be enzootic in snow buntings, and clinical cases could have occurred due to the stress associated with the captivity and handling for the research project.[2]

No treatment attempted on symptomatic birds proved to be successful in the described cases. As of today, only supportive care and treatment are indicated, but no specific treatment of avipoxvirus infection exists.[22,23] Management of an avipoxvirus epornitic in a captive collection should rely mainly on prophylaxis[19,22] as well as isolation of symptomatic birds. In the episode presented here, birds with lesions suggestive of avian poxvirus were isolated from the group. Other prophylaxis measures could include protection of the aviaries from vectors (indoor aviaries, mosquito nets).

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virus), and clade C (mainly psittacinepox virus). The arrows denote the new snow bunting avipoxvirus sequence reported in this article. The length of each horizontal bar indicates the amount of evolution as measured by substitution per site. The trees were made using the Juke–Cantor substitution model using a bootstrap setting of 1,000.

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