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Phylodynamic and phylogeographic reconstruction of beak and feather disease virus epidemiology and its implications for the international exotic bird trade

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Abstract

Beak and feather disease virus (BFDV) infects domestic and wild psittacine species and is able to cause progressive beak, claw and feather malformation and necrosis. In addition to having an impact on the health and welfare of domesticated birds, BFDV represents a significant threat to wild endangered species. Understanding the epidemiology, dynamics, viral migration rate, interaction between wild and domestic animals and the effect of implemented control strategies is fundamental in controlling the spread of the disease. With this in mind, a phylodynamic and phylogeographic analysis has been performed on a database of more than 400 replication-associated protein (Rep) gene (ORF1) sequences downloaded from Genbank including some recently generated sequences from fifteen samples collected in Namibia. The results allowed us to reconstruct the variation of viral population size and demonstrated the effect of enforced international bans on these dynamics. A good correlation was found between viral migration rate and the intensity of animal trade between regions over time. A dominant flux of viral strains was observed from wild to domestic populations, highlighting the directionality of viral transmission and the risk associated with the capturing and trade of wild birds. Nevertheless, the flow of viruses from domestic to wild species was not negligible and should be considered as a threat to biodiversity. Therefore, considering the strong relationship demonstrated in this study between animal trade and BFDV viral fluxes more effort should be made to prevent contact opportunities between wild and domestic populations from different countries in order to control disease spread.

KEYWORDS
BFDV, evolution, molecula epidemiology, phylodynamics, trade

1 | INTRODUCTION

Beak and feather disease virus (BFDV) is a member of the family *Circoviridae*, genus *Circovirus* and is characterized by a single-stranded circular DNA genome (ssDNA) of approximately 2 kb. Two major open reading frames (ORFs), ORF1 and ORF2, encode the viral replication-associated protein (Rep) and the major structural capsid protein (CP), respectively. BFDV causes psittacine beak and feather disease (PBFD), an often fatal disease that can have a peracute, acute or chronic course, in several psittacine birds (Fogell et al., 2016b, 2018). The most common signs and lesions in the infected bird are depression, lethargy, anaemia and abnormal feather growth. In some species, deformities to the beak and claws can also occur (Pass & Perry, 1984; Todd, 2004). Although affected birds may live for many years, more frequently, death ensues resulting from secondary infections due to BFDV-induced immunosuppression (Latimer et al., 1991). BFDV is highly infectious, and transmission can occur both vertically and horizontally, especially in crowded captive environments like breeding facilities, where environment and fomite contamination is a likely infection source (Raidal et al., 1993). Although officially described for the first time in the 1970s, the viral origin and the disease are probably much more ancient since historical documents, and previous studies describe clinical signs and lesions compatible with PBFD already in the late 19th century (Ashby, 1907; Das et al., 2016; Harkins et al., 2014; Todd, 2004). Since the first report, BFDV has now been detected at a global level in an increasing number of host species; a phenomenon likely driven by the trade of many bird species as pets.

Besides posing a major threat to global biodiversity particularly in African, Asian and South American countries, unsustainable and/or illegal wildlife trade is a menace for importing countries as well, as it is a significant pathway for the introduction of invasive species through escape or voluntary release. Infectious diseases can also be introduced through this route and can affect both wildlife conservation and livestock health and productivity (e.g. avian influenza) (Cardador et al., 2017; Ribeiro et al., 2019). For this reason, wildlife trade has been the subject of several bans in many countries.

Advances in sequencing technologies and decreasing costs have led to a significant increase in the available molecular epidemiology data on BFDV. This has allowed for an insight into host species and countries for which data was previously lacking. However, the data available is still only partial and biased, especially from low-income countries. It is thus of interest to update current knowledge and investigate BFDV migration patterns at a worldwide level, evaluating the effect of trade policies and bans on its dynamics by benefitting from increasing data resolution and accuracy. Such updates in knowledge could favour the formulation and assessment of government policies aimed at reducing the risk of BFDV importation, especially in countries where it can pose a major threat to wildlife conservation and to the livelihoods of commercial aviculturists (Heath et al., 2004). Based on these premises and to fill this gap, this study was undertaken to detect and molecularly characterize, for the first time, the circulation of BFDV in Namibia. In addition, a phylodynamic and phylogeographic analysis, based on a large collection of available BFDV sequences collected at the global level from both captive and wild birds, was performed to reconstruct the migratory, evolutionary and population dynamic patterns of BFDV.

2 | MATERIAL AND METHODS

A total of 103 cloacal swabs samples were collected by private veterinarians from asymptomatic and sick companion and wild birds in different areas of Namibia between April and October 2021 (Table 1). The swab samples were stored at 4°C until arrival at the laboratory. Total genomic DNA was extracted from the cloacal swabs using the High Pure Viral Nucleic Acid Kit (Hoffman-La Roche, Switzerland) with an elution volume of 100 μl following the manufacturer's instructions. The presence of BFDV-specific DNA was confirmed by PCR using primers: 2F (forward) 5′-AACCCTACAGACGGCGAG-3′ and 4R (reverse) 5′-GTCACAGTCCTCCTTGTACC-3′, which amplifies a fragment of 814 base pair (bp) of ORF1 (Ypelaar et al., 1999). The PCR reaction conditions consisted of 5 μl of extracted DNA in a final reaction volume of 25 μl containing a final concentration of 1.25 mM MgCl$_2$, 1× PCR buffer, 0.2 mM dNTPs, 10 pmol of each primer and 2.5 U of Taq DNA polymerase (Qiagen, Germany). All the reactions were performed with the following thermal profile: initial denaturation at 95°C for 5 min and then 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s and elongation at 72°C for 45 s, followed by a final elongation at 72°C for 10 min. Of the 103 samples tested for BFDV using the conventional PCR and primers F2 and R4, a total of 15 samples were positive (Table 1). The PCR amplicons were purified using a Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced commercially by LGC Genomics (Berlin, Germany). The sequences of the positive samples were submitted to GenBank under accession numbers ON013735-ON013749. The sequences were edited and assembled using the Staden software package version 2.0.0b8. Multiple sequence alignments were performed using the ClustalW algorithm implemented in the BioEdit software package version 7.2.6 to compare the partial ORF1 gene sequences of the isolates.

2.1 | Dataset preparation

BFDV ORF1 sequences spanning the same region obtained in the present study were downloaded from GenBank if the sampling country, host and date were available (Supplementary Table S1). All sequences were aligned using MAFFT (Standley, 2013) and their quality was evaluated. Poorly aligned sequences, those displaying unknown bases, premature stop codons, or frameshift mutations were excluded from further analysis. Recombination analysis was performed as described by Franzo et al. (2021), and recombinant strains were also excluded from further analysis. The absence of residual recombination signals was assessed using GARD (Kosakovsky Pond et al., 2006). The presence of adequate phylogenetic and temporal signals was tested using

TABLE 1 Summary table reporting the count and location of sampled individuals for each species

Species	Number	Positive BFDV	Location
Budgerigar (*Melopsittacus undulates*)	14	3	Windhoek
Dove (*Columba livia domestica*)	4	0	Windhoek
Vulture (*Gyps africanus*)	18	0	Etosha – Mangheti
Citron cockatoo (*Cacatua sulphurea citrinocristata*)	2	0	Windhoek
Goffin cockatoo (*Cacatua goffiniana*)	1	0	Walvis Bay
Peacock (*Pavo cristatus*)	1	0	Windhoek
Flamingo (*Phoenicopterus roseus*)	1	0	Lüderitz
Rosy Love bird (*Agapornis roseicollis*)	5	1	Windhoek – Walvis Bay
Cockatiel (*Nymphicus hollandicus*)	5	0	Walvis Bay
Parrot (*Poicephalus* spp.)	11	1	Windhoek – Walvis Bay
Plum-headed parakeet (*Psittacula cyanocephala*)	1	0	Walvis Bay
Alexandrine parrot (*Psittacula eupatria*)	2	0	Windhoek
Indian Ringneck (*Psittacula krameri*)	8	4	Windhoek- Walvis Bay
Conure (*Aratinga spp.*)	10	3	Windhoek- Walvis Bay
African grey (*Psittacus erithacus*)	7	1	Windhoek – Walvis Bay – Swakopmund
Lilac-breasted roller (*Coracias caudatus*)	1	0	Walvis Bay
Grey loerie (*Corythaixoides concolor*)	2	0	Walvis Bay
Lineolate parakeet (*Bolborhynchus lineola*)	1	0	Walvis Bay
Pacific parrotlet (*Forpus coelestis*)	1	0	Walvis Bay
Illiger macaw (*Primolius maracana*)	1	0	Walvis Bay
Pionus (*Pionus spp.*)	2	1	Walvis Bay
Kakariki (*Cyanoramphus spp.*)	5	1	Walvis Bay

Note: The number of BFDV-positive subjects is also annotated.

the likelihood mapping approach implemented in IQ-Tree (Nguyen et al., 2015) and the TempEst (Rambaut et al., 2016) programs, respectively.

2.2 | Viral population dynamics and phylogeography

BFDV population parameters, including time to the most recent common ancestor (tMRCA), evolutionary rate and population dynamics variation over time were jointly estimated in a Bayesian fashion using the serial coalescent approach implemented in BEAST 1.10.4 (Suchard et al., 2018). The nucleotide substitution model was selected based on the Bayesian Information Criterion (BIC) calculated using JModelTest2 (Darriba et al., 2012), while the molecular clock model was chosen based on Bayesian factor (BF) calculation obtained by estimating the marginal likelihood of the evaluated models using the path sampling (PS) and stepping stones (SS) methods (Baele et al., 2012). The nonparametric Skygrid (Hill & Baele, 2019) model was selected to reconstruct the trend of the relative genetic diversity (i.e. effective population size × generation time; Ne × t) over time. Strain migration among countries was reconstructed using the discrete state phylogeographic

approach described by Lemey et al. (2009) considering each collection country as a strain trait. The Bayesian stochastic search variable selection (BSSVS) was also implemented to allow for the construction of a BF test and identify the most parsimonious description of the phylogeographic diffusion process. Symmetric versus asymmetric migration models were compared through calculation of the marginal likelihood using the PS and SS methods, as previously described. All parameters were jointly estimated using a 200 million generation Markov Chain Monte Carlo (MCMC) chain, sampling the population parameters and trees every 20,000 generations. Run performances were summarized and evaluated using Tracer 1.7 after removing the first 20% of the data as burn-in. Run results were accepted only if the estimated sample size was higher than 200 and the mixing and convergence, evaluated by visual inspection of the run's trace, were adequate. A maximum clade credibility (MCC) tree was obtained summarizing over the tree posterior distribution using the TreeAnnotator suite of the BEAST package. The viral spreading was visualized using SPREAD3 (Bielejec et al., 2016). The same software allowed for the identification of the statistically supported migration rates between country pairs. The significance level was set to BF > 10 for all considered analyses. Additional summary statistics and graphics were generated using R (Team, 2014) and specific libraries (Ginestet, 2011; Yu et al., 2017).

2.3 | Wild–domestic interaction

BFDV circulation in domestic and wild species and effective contact between these has been described (Fogell et al., 2016a). Nevertheless, the respective contribution, the intensity and directionality of the flux have never been formally assessed. For this purpose, information on the wild-captive status of the birds from which sequences originated was obtained from the literature. When no or inaccurate data were available, sequences were excluded from the study. A structured coalescent approach was implemented on the new dataset. Briefly, according to this model, the considered population was divided into two demes (i.e. wild and domestic birds), which can be considered as different islands, characterized by their own population size and interconnected by a certain migration rate among them (de Maio et al., 2015; Vaughan et al., 2014). The Marginal Approximation of the Structured COalescen T (MASCOT) (Müller et al., 2018) implemented in BEAST2 (Bouckaert et al., 2019) was selected. Substitution and clock models were selected as described in the previous section. The parameters were estimated by performing a 200 million generation MCMC run, sampling parameters and trees every 20,000 generations. Obtained posterior estimations were managed as described in the previous section.

3 | RESULTS

3.1 | BFDV diagnosis: Clinical signs, molecular diagnosis and genetic characterization

Fifteen out of 103 (14.6% CI = 9.06–22.67%) samples tested positive for BFDV by PCR. Of these, four were Indian Ringneck (*Psittacula krameri*), three Budgerigars (*Melopsittacus undulates*) and three Conures (*Aratinga* spp.), while one positive sample was detected for each of the following: Rosy Love bird (*Agapornis roseicollis*), Parrot (*Poicephalus* spp.), African grey (*Psittacus erithacus*), Pionus (*Pionus* spp.) and Kakariki (*Cyanoramphus* spp.). Of the positive birds, the Budgerigar displayed generalized feather disease, featured by feather dysplasia, haemorrhages and dystrophy, while one of the Conures was affected by abdominal distension, subcutaneous and subserosal oedema, serosal haemorrhages on multiple organs, hydropericardium, hepatomegaly and splenomegaly. No clinical signs or lesions were observed in the other subjects. The complete sequence of the ORF1 was obtained for all positive samples. Phylogenetic analysis on Namibian strains revealed the presence of two independent clusters, including 11 (Cluster A) and 4 (Cluster B) sequences, characterized by a 6.23% raw genetic distance between groups. In Cluster A, the closest relationship with the Namibian strains was observed with viruses collected between 2017 and 2018 in Saudi Arabia followed by Italy and South Africa. In contrast, no close relative was identified for the Clade B strains (Figure 1 and Supplementary Figure S1).

3.2 | Phylodynamics and phylogeographic analysis of Namibian strains within the international context

The phylodynamic analysis revealed a high evolutionary rate of BFDV in the genomic region considered (i.e. 7.86×10^{-4}; 95HPD: 5.37×10^{-4}–1.05×10^{-3}). The estimated tMRCA was 1,939.07, 95HPD: 1,918.13–1,958.21. Based on these estimations, the likely introduction of Cluster A into Namibia occurred, approximately, in 2010. In the absence of closely related strains from other regions, less recent introduction was estimated for Cluster B. The reconstruction of the viral population dynamics highlighted a progressive increase in the global viral population size from tMRCA to the late 1960s when a slowdown of the process was observed until the late 1990s. The following phase was essentially characterized by stabilization and then a decline, starting from the new millennium, although with some minor fluctuations (Figure 2).

The pattern of global viral spread over time was also evaluated (Figure 3). After a likely origin in Australia, the virus apparently spread to Europe (most likely Poland) and Mauritius in the 1980s. Following the first introduction, European countries represented a major source of viral spread during the 1990s to other European, Asian and African countries. After the first introduction into Asia, some countries contributed to local dispersal, particularly Iran, Bangladesh and Thailand from 2000 onwards. In the meantime, Australia continued to contribute to BFDV introduction especially in Asia and other regions of Oceania. Finally, strain migration to South America is seen as originating from both Europe and Australia. Although rarer, within Africa exchanges, mediated by Saudi Arabia, were also observed. Accordingly, the evaluation of adequately supported migration rates (i.e. BF > 10) revealed a pattern essentially characterized by two main nuclei of viral dispersal, Europe and to a lesser extent Australia (Figure 4). Australia, besides being the initial source of viral emergence, contributed mainly to a shorter range dispersal to other countries in Oceania and Asia. Europe was the main source for worldwide dispersal to Asia, Africa and South America (Figure 4). Finally, significant within-continent migration rates were identified in Asia and Europe, contributing to local dispersal.

3.3 | Wild–domestic interaction

The structured coalescent analysis allowed the respective deme sizes of BFDV circulating in wild and domestic populations and the strain migration among them to be estimated. Overall, the viral population size was 18.52 times bigger in the wild population compared to the domestic one. Similarly, the intensity of the viral flux from the wild to the domestic environment was 37.11 times greater than the other way around. Accordingly, the MCC tree highlighted a much more frequent transition from wild to domestic species than vice versa (Figure 5).
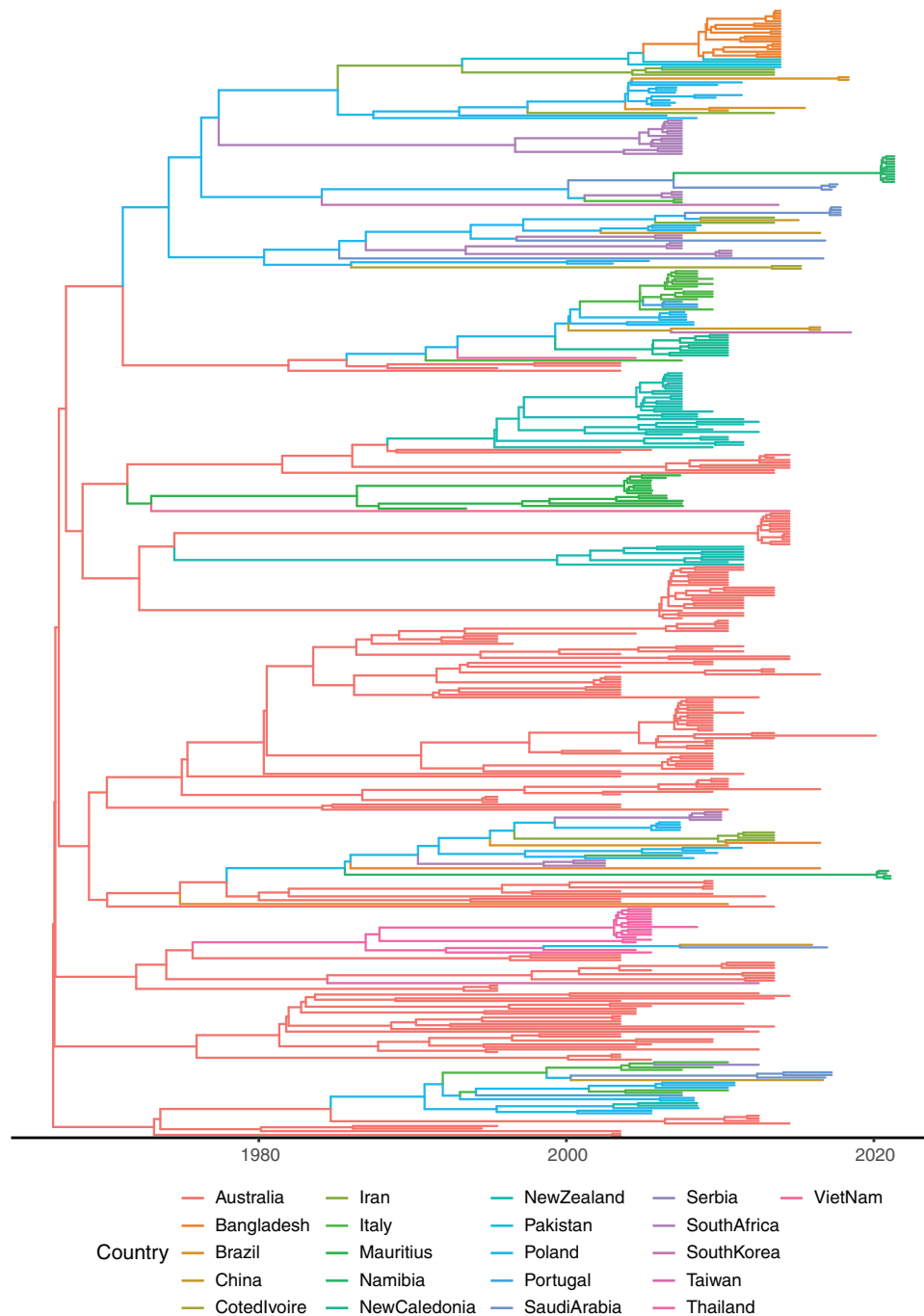
FIGURE 1 Time scaled, maximum clade credibility (MCC) trees of BFDV strains. The tree branches have been colour coded according to the country location predicted with the highest posterior probability

4 | DISCUSSION

Many factors are contributing to new and old disease emergence/re-emergence, epidemiological changes and spread, and most of them are caused, at least to a certain extent, by humans (Lindahl & Grace, 2015). Among these, globalization, international travel and trade contribute to pathogen dispersal (Manuja et al., 2014; Travis et al., 2011). The wildlife trade, in combination with human encroachment into natural habitats or expansion of wildlife into human settlements

and habitations, is considered the main risk for disease emergence and/or spread (Travis et al., 2011).

The psittacine order comprises a broad range of wild avian species some of which are kept for ornamental purposes. The boundary between wild and domesticated bird categories is hard to define since wild individuals are often captured and sold as companion animals and domesticated individuals are sometimes accidentally or intentionally released into the wild. Such scenarios represent clear risks from an infectious disease perspective since infectious diseases can be
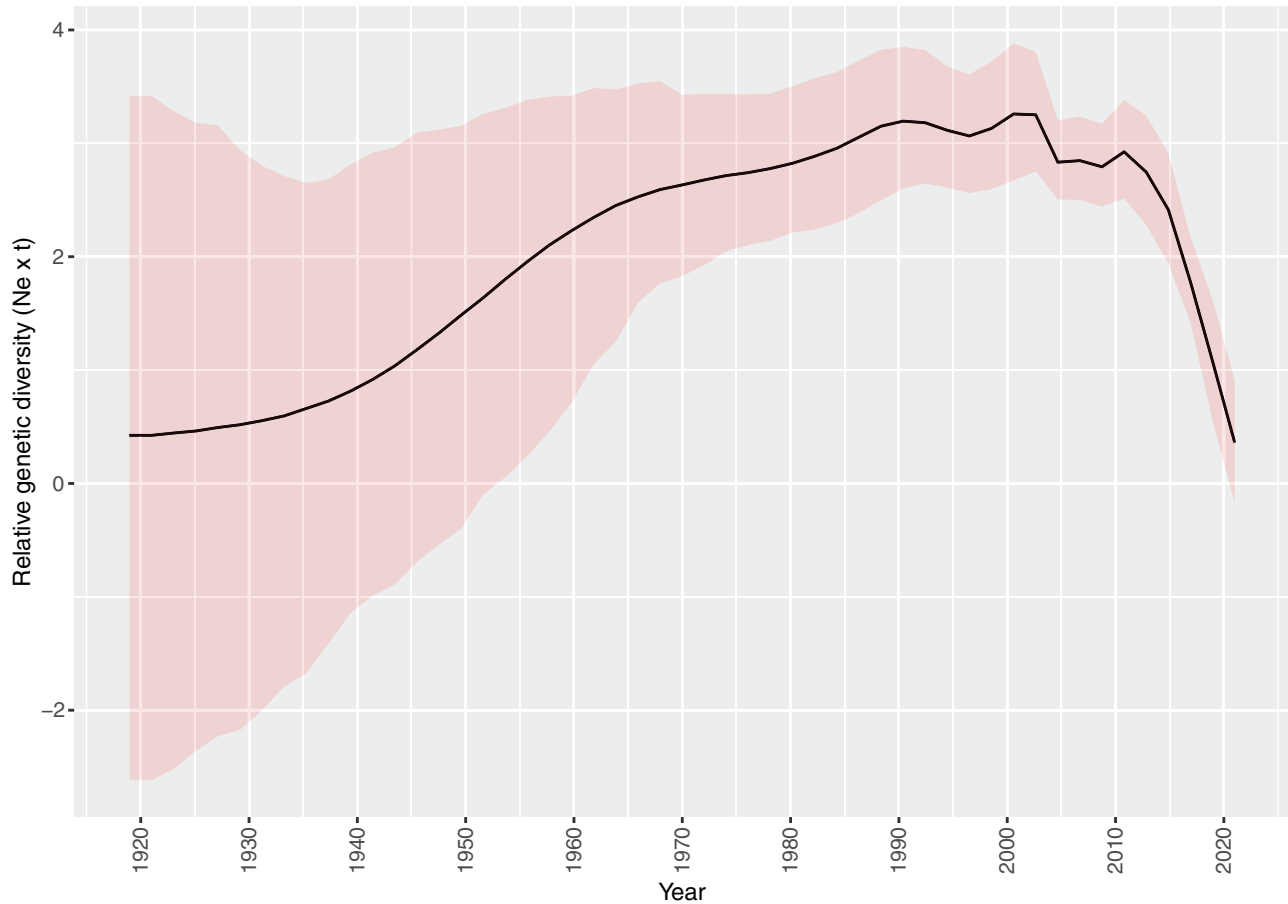
FIGURE 2 Mean relative genetic diversity (Ne × t) of the worldwide BFDV population over time

maintained in the wild where no effective control strategies are applied and represent a source for new introductions into domestic hosts (Manuja et al., 2014). On the other hand, contact with domestic animal infections can represent a threat to wild species, especially endangered ones (Daszak et al., 2000; Gortazar et al., 2015). BFDV is an excellent example in this sense since both captive and wild individuals can be infected and complex epidemiological links can be created between these populations which can have global repercussions due to intense trade, both legal and illegal (Fogell et al., 2016a, 2018; Harkins et al., 2014; Katoh et al., 2010).

The present study investigated the main epidemiological pattern of BFDV at a global level, in addition to expanding the current knowledge of its circulation in the largely neglected areas of Africa, Namibia in particular. The high evolutionary rate of BFDV, similar to other ssDNA viruses (Duffy et al., 2008) was confirmed in the present study and testifies the potential of this virus to adapt to new environmental conditions. Accordingly, a relatively recent tMRCA was estimated, which partially conflicts with anecdotal and historical evidence of more ancient origins (Ashby, 1907; Das et al., 2016; Harkins et al., 2014; Todd, 2004). Such a tendency of overestimating the evolutionary rate of rapidly evolving viruses (and thus underestimating the tMRCA) is a documented phenomenon known as Time-Dependent Rate Phenomenon in viruses (TDRP) (i.e. viral evolutionary rates appear to

vary over time, decreasing with the timescale of rate measurement) (Aiewsakun & Katzourakis, 2016; Ho et al., 2011).

Nevertheless, such an approach has been repeatedly shown to be reliable in modelling and reconstructing virus history, evolution and dynamics over a shorter time span (Holmes & Grenfell, 2009; Volz et al., 2009, 2013). In fact, in accordance with historical evidence, the origin of BFDV was predicted in Australia, which acted as the first nucleus of viral dispersal to Europe and neighbouring countries. Thereafter, viral spread affected all continents in the following decades. Because of the inability of psittacine birds to migrate over long distances, the impact of bird trading appears to be pivotal in viral dispersal. Unfortunately, a precise description of this trading is hard to obtain because legal and registered trading is often accompanied by illegal trading, for which trade routes and volumes are difficult to quantify (Reino et al., 2017). Also, from a viral molecular epidemiological perspective, the available data are poor and the sequences are the result of uncoordinated convenience samplings, rather than a planned investigation. For this reason, a commonly sequenced region of the ORF1 was selected for the analysis, trying to maximize the available information and global representativeness. Moreover, since it is well known that circoviruses display a high recombination rate, especially in the intergenic region (Lefeuvre et al., 2009), this choice prevented the exclusion of recombinant strains and minimized the risk of the inclusion of undetected
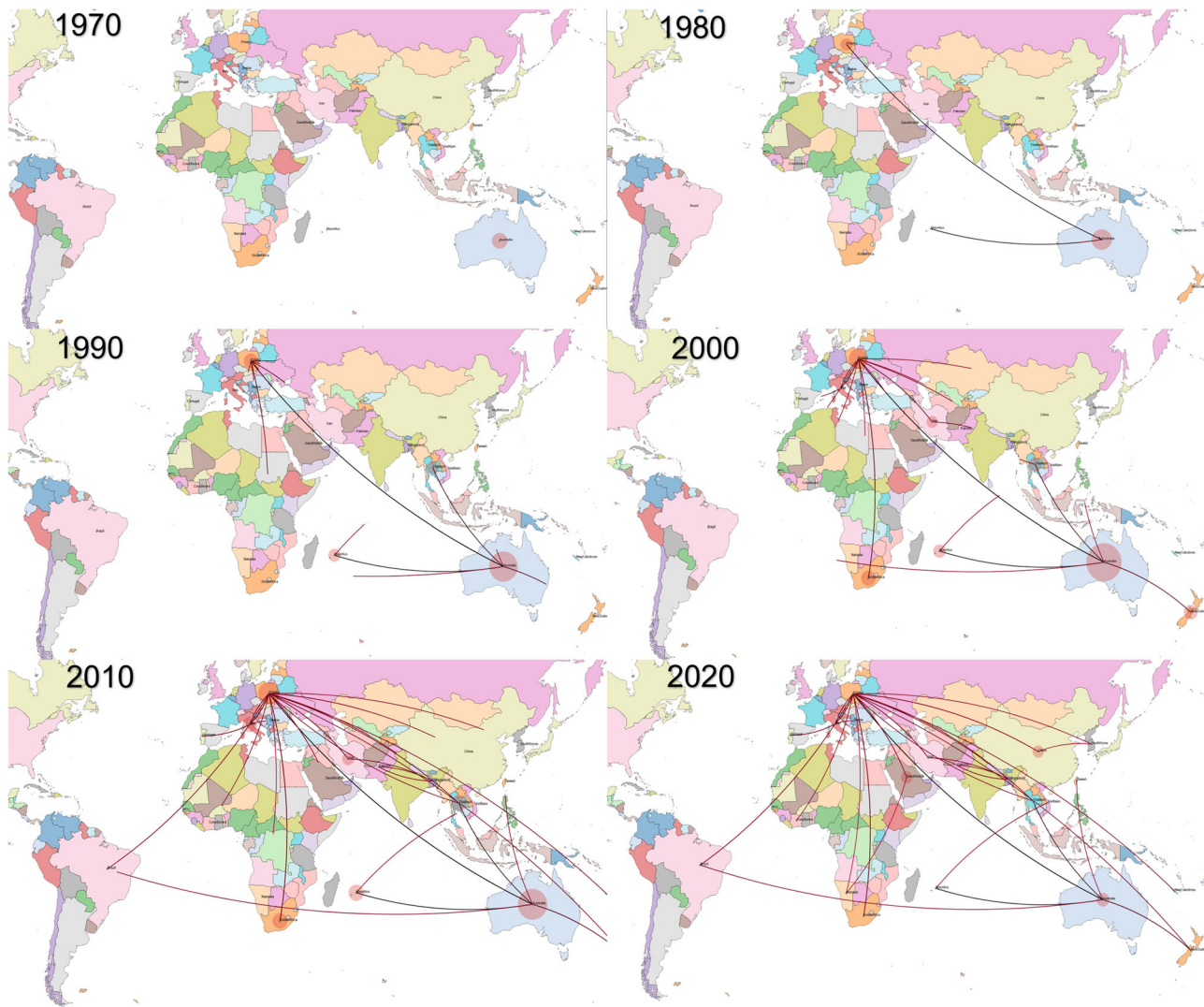
FIGURE 3 Phylogeographic reconstruction of BFDV migration over time. The size of the polygons around a sampling location is proportional to the number of lineages that maintain that location, while edges have been colour-coded from the most ancient (black) to the most recent (red) migration

recombination events, which is known to negatively affect the parameter estimation and results accuracy and reliability. At the same time, formal statistical tests demonstrated that the considered region had a phylogenetic and temporal signal that was adequate to perform the present analyses. Nevertheless, despite our efforts, sequencing activities and output are much higher in high-income countries, which justify the apparent dominant viral flux from high-to-low income countries. Moreover, the absence of relevant metadata including date and country of collection, poor sequence quality or the sequencing of non-standardized region of the virus led to the exclusion of different BFDV strains, and thus countries, from the current analysis. Therefore, some epidemiological links among countries could have been missed in the present analysis, inviting caution in the interpretation of the results.

Nevertheless, the overall epidemiological pattern appears clear. Europe is a major nucleus of both BFDV importation and exportation. The higher statistical support in favour of the symmetric migration model (i.e. same rate of strain importation and exportation between

country pairs) implemented in the phylogeographic analysis confirms this evidence and testifies that the viral exchange was at least bidirectional, which is in agreement with experts opinion, which considers Europe both as an importer and exporter of birds at a global scale (Ribeiro et al., 2019). The role of African, South-East Asian and South American countries as exporters of wild birds to the Northern hemisphere (Reino et al., 2017) is also supported by the selected model. More recently, exotic bird importation has increased from African, Middle Eastern and Asian countries, most likely because of the emergence of their economies. Accordingly, their increasing role in the spread of BFDV since the late 1990s – beginning of the new millennium was observed. Redirection of trade from developed countries, where resources to manage transboundary movement of animal diseases are in place and social awareness is high, towards developing countries, which are less well equipped to deal with such movements, may contribute to increasing introduction risks and consequent impacts on native species (Ribeiro et al., 2019). Strong evidence on the
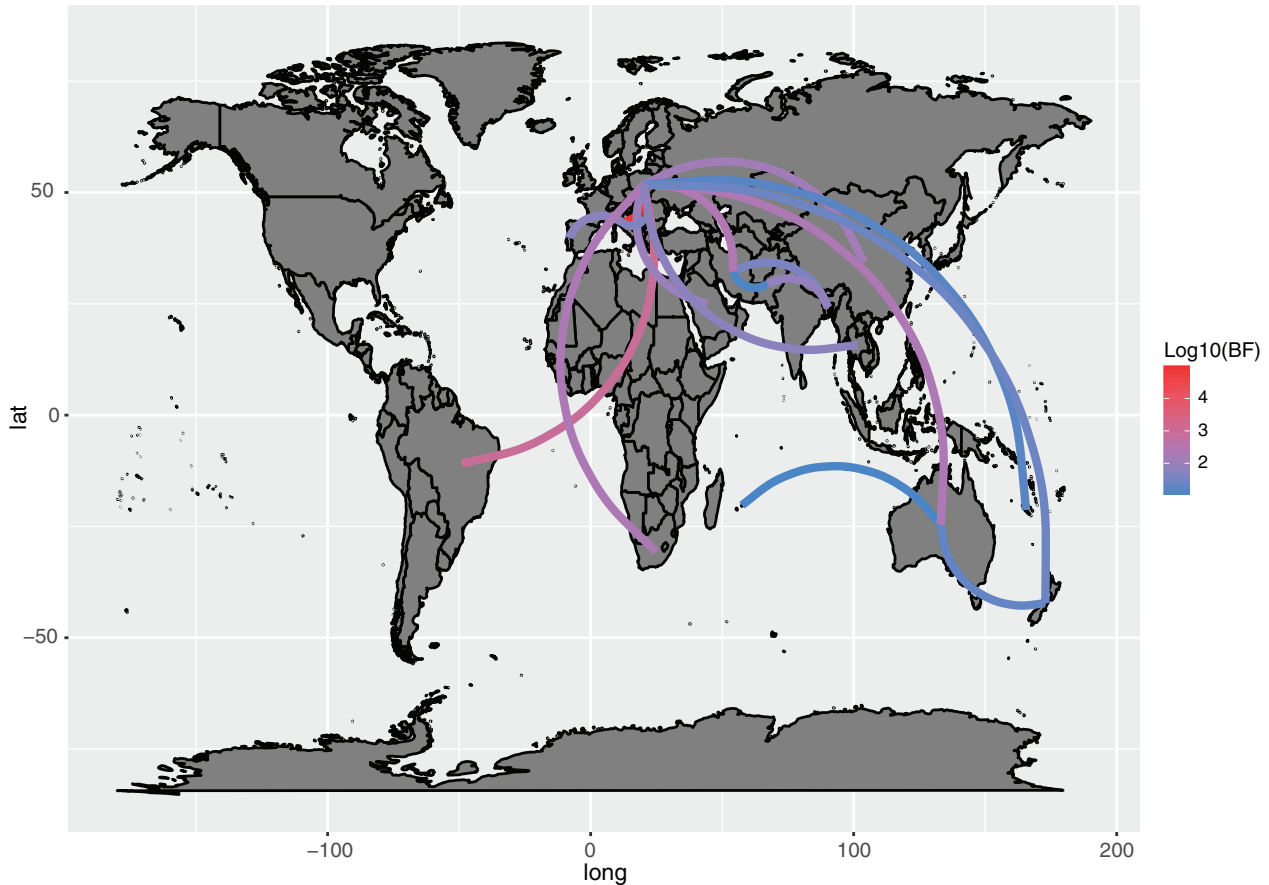
FIGURE 4 Well-supported migration paths (i.e. BF > 10) among countries. Connections are depicted as edges whose colour is proportional to the logarithm of the BF of the inferred link. The location of each country has been matched with its centroid

implications of the bird trade emerges from the analysis of viral population dynamics in this study. In fact, a quick increase in BFDV geographic distribution and population size was observed in the first decades after its tMRCA, when limited constraints to legal and illegal trade were applied. Of note, the first decline in the expansion rate occurred in the late 1990s, when a substantial stabilization of the viral population was estimated. This period followed the Wild Bird Conservation Act, which was enacted by the United States in 1992 (Cardador et al., 2017). Besides the direct impact in North America, this ban had a profound effect at a worldwide level. For example, although a similar number of parrots (2.1 million individuals; 0.16 million per year) continued to be traded worldwide, the EU countries acquired a more dominant role in international trade. Imports also increased in Mexico, South-America and across Southeast Asia (Cardador et al., 2017), which perfectly fit with the timing of viral migration toward these regions reconstructed by our phylogeographic analysis.

A second major ban, the Wild Bird Declaration, was imposed by the EU in 2005, first as a temporal measure to prevent the spread of avian flu and other diseases, and has been indefinitely extended since 2007, leading to imports reaching almost zero in EU countries (Cardador et al., 2017). At the same time, the risk of unintended psittacine bird introduction decreased worldwide (Cardador et al., 2017). As expected, based on the importance of Europe in the spread of BFDV,

a dramatic decrease in viral population size has been observed since 2005. Overall, a good match between the reconstructed phylogeographic BFDV dispersal and estimations in the change in psittacine bird introduction risk in different parts of the world in response to the mentioned bans (Cardador et al., 2017) was observed. For example, an increased risk was reported for Namibia following the American ban in 1992, in the same period when BFDV strain introduction was predicted in the present study. Of note, while the source of Clade B is more obscure, strong support was obtained for Clade A originating from Saudi Arabia, one of the countries whose involvement in bird trade increased in the corresponding period (Mohamed et al., 2021). Overall, the available data provide a coherent picture, which testifies a strong relationship between bird trade and viral dispersal. The structured coalescent approach implemented in the present study provides further support and objective quantification of the role of wild and captive birds. Wild birds not only hosted a broader viral population, largely expected based on the population which is an order of magnitude larger than the captive population, but also acted as a source of strain introduction in the domestic environment. Bird mixing in origin or destination markets and shops might further contribute to the efficiency of the spreading process (Bezerra-Santos et al., 2021; Karesh et al., 2007; Martens et al., 2020; van den Berg, 2009). Even though less relevant numerically, the opposite flow, from domestic to wild species, was
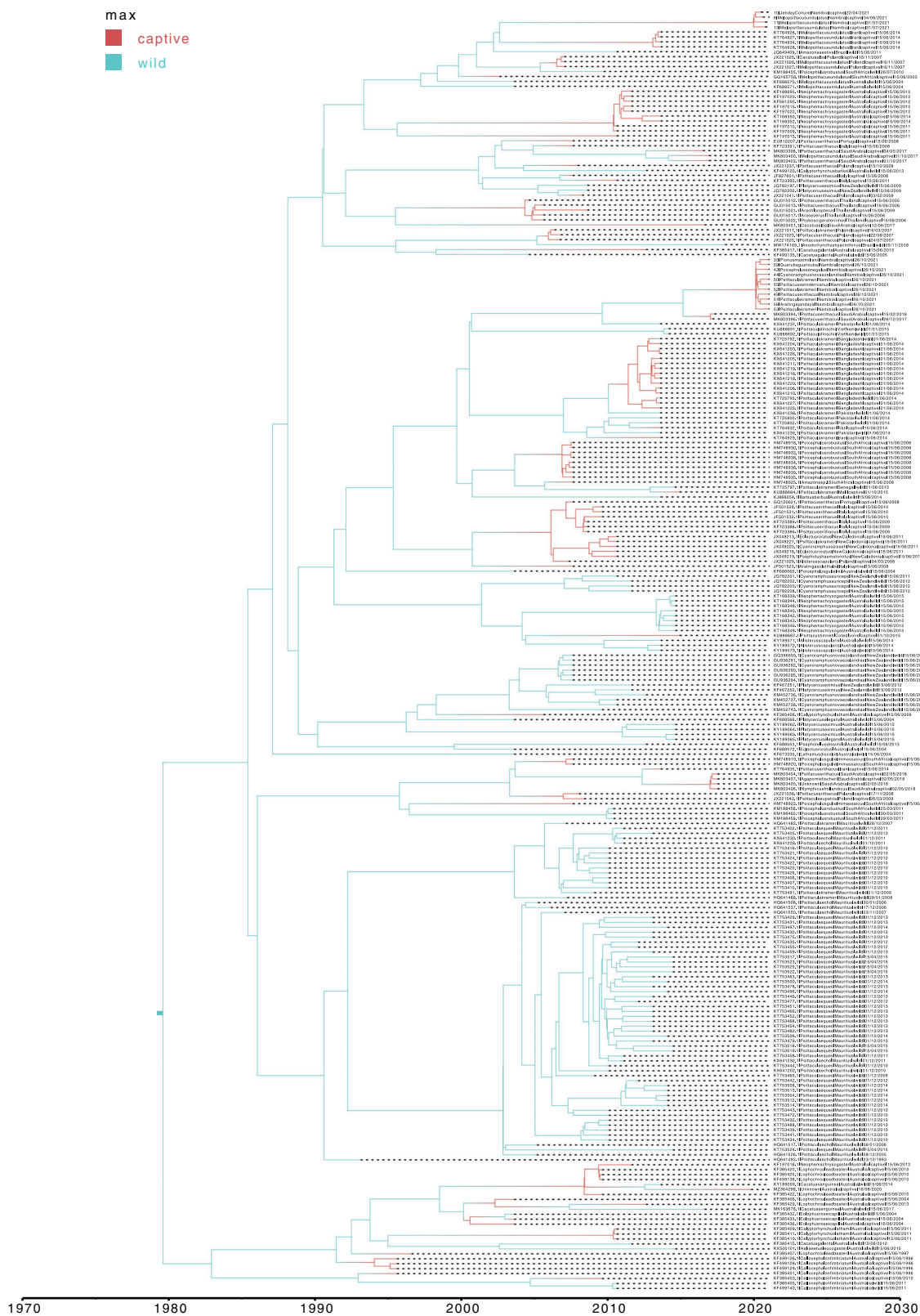
FIGURE 5 Time scaled, maximum clade credibility (MCC) trees of BFDV strains, reconstructed using the structured coalescent approach. The tree branches have been colour-coded according to the host (wild or domestic) predicted with the highest posterior probability

still relevant and cannot be ignored because of the potential relevance in the protection of endangered species (Das et al., 2015). Therefore, considering the strong relationship herein demonstrated between animal trade and viral fluxes and in light of the remarkable success of international bans in constraining bird trade, especially illegal trade, more efforts should be made to prevent and constrain effective contact opportunities between bird populations in different countries and between wild and domestic population. Such an approach, to be effective, would require an international collaborative effort and involve not only legislative and repressive measures but also investment in the education and sustenance of bird sellers and villagers capturing wild birds, for which such trade often represents an essential income.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as all samples originated from the routine diagnostic activity and no experimental procedure was performed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Genbank at https://www.ncbi.nlm.nih.gov/nucleotide/

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