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Contributed Paper

Trade and conservation implications of new beak and feather disease virus detection in native and introduced parrots

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Abstract: *Psittacine beak and feather disease (PBFD), caused by Beak and feather disease virus (BFDV), has spread rapidly around the world, raising concerns for threatened species conservation and biosecurity associated with the global pet bird trade. The virus has been reported in several wild parrot populations, but data are lacking for many taxa and geographical areas with high parrot endemism. We aimed to advance understanding of BFDV distribution in many data-deficient areas and determine phylogenetic and biogeographic associations of the virus in 5 parrot species across Africa, the Indian Ocean islands, Asia, and Europe and focused specifically on the highly traded and invasive* Psittacula krameri*. Blood, feather, and tissue samples were screened for BFDV through standard polymerase chain reaction. Isolates obtained from positive individuals were then analyzed in a maximum likelihood phylogeny along with all other publically available global BFDV sequences. We detected BFDV in 8 countries where it was not known to occur previously, indicating the virus is more widely distributed than currently recognized. We documented for the first time the presence of BFDV in wild populations of* P. krameri *within its native range in Asia and Africa. We detected BFDV among introduced* P. krameri *in Mauritius and the Seychelles, raising concerns for island endemic species in the region. Phylogenetic relationships between viral sequences showed likely pathways of transmission between populations in southern Asia and western Africa. A high degree of phylogenetic relatedness between viral variants from geographically distant populations suggests recent introductions, likely driven by global trade. These findings highlight the need for effective regulation of international trade in live parrots, particularly in regions with high parrot endemism or vulnerable taxa where* P. krameri *could act as a reservoir host.*

Keywords: infectious disease, invasive alien species, pet trade, reservoir host, vulnerable taxa

Implicaciones para el Mercado y la Conservación de la Detección del Nuevo *Virus de la Enfermedad de Plumas y Pico* en Loros Nativos e Introducidos

Resumen: *La enfermedad de plumas y pico de los psitácidos (PBFD, en inglés), causada por el* virus de la enfermedad de plumas y pico *(BFDV, en inglés), se ha esparcido rápidamente en todo el mundo, ocasionando*

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***Article impact statement**: Beak and feather disease virus is newly detected in 8 countries; thus, regulation of international trade in live parrots is crucial.*
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mayor preocupación por la conservación de especies amenazadas y la bioseguridad asociada con el mercado mundial de aves de compañía. El virus ha sido reportado en varios poblaciones silvestres de loros, pero los datos son muy pocos para muchos taxones y áreas geográficas con un alto endemismo de loros. Buscamos avanzar el entendimiento de la distribución del BFDV en muchas áreas deficientes de datos y determinar las asociaciones filogenéticas y biogeográficas del virus en cinco especies de loros en África, las islas del océano Índico, Asia y Europa, enfocándonos específicamente en la especie invasiva y muy comercializada Psittacula krameri. *La presencia de BFDV en las muestras de sangre, plumas y tejido fue examinada por medio de un PCR estándar. Los casos aislados que se obtuvieron de individuos positivos fueron analizados después en una probabilidad máxima de filogenia junto con todas las otras secuencias de BFDV disponibles públicamente a nivel mundial. Detectamos el BFDV en ocho países en los que no se tenía registrado algún caso previo, lo que indica que el virus tiene una distribución más amplia de lo que se reconoce actualmente. Documentamos por primera vez la presencia del BFDV en poblaciones silvestres de* P. krameri *dentro de su distribución nativa en Asia y en África. Detectamos el BFDV entre poblaciones introducidas de* P. krameri *en Mauricio y en las Seychelles, lo que incrementa la preocupación por las especies endémicas de las islas en la región. Las relaciones filogenéticas entre las secuencias virales mostraron vías posibles de transmisión entre las poblaciones en el sur de Asia y el oeste de África. Un alto grado de relación filogenética entre las variantes virales de poblaciones distantes sugiere introducciones recientes, probablemente a causa del mercado mundial. Estos resultados resaltan la necesidad de una regulación efectiva del mercado internacional de loros, particularmente en regiones con un alto endemismo de loros o taxones vulnerables en donde* P. krameri *podría fungir como hospedero reservorio.*

Palabras Clave: enfermedad infecciosa, especie foránea invasora, hospedero reservorio, mercado de mascotas, taxones vulnerables

摘要: *鹦鹉喙羽病是由喙羽病病毒 (beak and feather disease virus, BFDV)引起的一种疾病, 已在世界范围内迅速传播, 并引起了人们对濒危物种保护和全球宠物鸟类贸易相关的生物安全问题的关注。目前已在多个野生鹦鹉种群中发现了该病毒, 但许多类群和鹦鹉特有种多的地区还缺少相关数据。本研究的目的在于了解数据缺乏地区的BFDV分布情况、确定非洲、印度洋岛屿、亚洲和欧洲五种鹦鹉中病毒的系统发育和生物地理关系, 特别关注贸易和入侵频发的红领绿鹦鹉 (Psittacula krameri) 。我们通过标准 PCR 流程检测鹦鹉的血液、羽毛和组织样品中是否存在BFDV病毒, 并将阳性个体中分离得到的病毒序列与全球所有公开发表的BFDV序列用最大似然法进行系统发育分析。我们在八个以前没有报道过存在BFDV的国家发现了该病毒, 表明病毒的分布比目前所了解的更广。我们还首次在亚洲和非洲的红领绿鹦鹉原生地的野生种群中发现了BFDV。被引入毛里求斯和塞舌尔群岛的红领绿鹦鹉中也检测到BFDV, 这引起了我们对该地区岛屿特有种的担忧。病毒序列间的系统发育关系显示了南亚和西非。来自地理距离较远种群的病毒变异株系统发育关系的高度相似性, 说明存在近期的引入, 这可能是受到全球贸易的推动。这些发现强调了有效管理活体鹦鹉全球贸易的必要性, 特别是在鹦鹉特有种多或是有易危类群的地区, 要注意红领绿鹦鹉可能作为储存宿主来传播病毒。【翻译: 胡怡思; 审校: 聂永刚】*

关键词: 传染病, 外来入侵种, 宠物贸易, 储存宿主, 易危类群

Introduction

The global spread of pathogens poses an increasing threat to biodiversity (Daszak et al. 2000) and has been linked to wildlife-population collapse and multiple species extinctions (Cunningham et al. 2017). Parrots are among the most threatened bird groups (Olah et al. 2016) and are susceptible to a number of infectious diseases (Ritchie 1995). Parrots are also among the most frequently traded birds listed on the appendices of the Convention on International Trade in Endangered Species (CITES) (Pain et al. 2006), and the pet trade has driven cross-border movements of over 19 million parrots since 1975 (CITES 2016). This movement has exacerbated the establishment of numerous introduced populations, most notably the highly invasive Rose-ringed Parakeet (*Psittacula krameri*), which has breeding populations in over 35 countries across 5 continents (Tayleur 2010; Menchetti et al. 2016).

Psittacine beak and feather disease (PBFD), caused by Beak and feather disease virus (BFDV), is a commonly reported infectious disease of captive parrots. First described in the 1970s (Pass & Perry 1984) in the South Pacific (Ritchie et al. 1989; Heath et al. 2004; Harkins et al. 2014), PBFD is thought to have post-Gondwanan origins due to the paucity of ancestral non-Australian clades and infrequent observations across other regions where parrot endemism is high, such as Africa and South America (Raidal et al. 2015). All psittaciformes are susceptible to infection (Sarker et al. 2014), and PBFD is typically characterized by chronic symmetrical feather abnormalities, dystrophy, and severe claw and beak deformities (Latimer et al. 1991; Bassami et al. 1998). The immunosuppressant nature of BFDV increases host susceptibility to secondary infection (Ritchie et al. 1989, 2003). The spread of BFDV may be facilitated by the global trade in live parrots (e.g., Varsani et al. 2011; Harkins et al. 2014) and its high environmental persistence and transmissibility between

closely related host species (Peters et al. 2014; Sarker et al. 2014). To date BFDV or PBFD have been recorded in 78 species and 5 subspecies (Fogell et al. 2016). Infection of parrots in captivity has been reported in at least 33 countries, whereas the virus occurs in comparatively few wild populations outside Oceania, where BFDV is believed to have originated (Raidal et al. 2015; Fogell et al. 2016).

Increasing reports of BFDV infections in wild populations, both native and introduced, including several populations of threatened species, have led to concerns over the conservation implications of the spread of infection (Kundu et al. 2012; Regnard et al. 2014; Jackson et al. 2015*a*). Although invasive populations and captive individuals of Rose-ringed Parakeets have tested positive for BFDV (Kundu et al. 2012; Julian et al. 2013; Sa et al. 2014), to date no BFDV screening of Rose-ringed Parakeets has been conducted on any free-living populations across their extensive native range (Fogell et al. 2016). The rapid adaptability and successful establishment of Rose-ringed Parakeets globally make it a high-risk reservoir host and vector for BFDV, particularly where its distribution overlaps with that of vulnerable species. These concerns have prompted actions, such as the eradication of Rose-ringed Parakeets on the island of Mahé, Seychelles, to minimize threats to the endemic Seychelles Black Parrot (*Coracopsis barklyi*). This eradication campaign was launched in 2013 in response to concerns over biosecurity (Seychelles Islands Foundation 2013), particularly in light of the similar BFDV-affected parakeet populations in Mauritius (Kundu et al. 2012).

Despite increasing surveillance effort over recent years (Fogell et al. 2016), there remains a paucity of information on BFDV distribution, notably in regions of high parrot endemism in Africa, Asia, and South America (Fogell et al. 2016) and from parrots seized from illegal trade. Insufficient knowledge of the distribution of the virus among native and introduced populations and within trade hampers understanding of the biogeography and origins of BFDV and the potential conservation impacts of PBFD, and impedes the development of effective approaches to prevent BFDV spread.

We aimed to determine the presence of BFDV in native and introduced wild parrot populations in data-deficient regions and taxa across 3 continents, and to establish phylogenetic and biogeographic associations of the virus among wild and captive populations and parrots in illegal trade based on viral sequence analysis. We screened samples obtained from native and introduced populations of parrots from Africa, Asia, and Europe of Seychelles Black Parrots, Mauritius Echo Parakeets (*Psittacula eques*), Grey-headed Parakeets (*Psittacula finschii*), Rose-ringed Parakeets, and Timneh Parrots (*Psittacus timneh*) for the presence of BFDV. We focused on the Rose-ringed Parakeet because of its potential to act as a reservoir host across its native and invasive range.

Methods

Wild Parrot Sampling

Blood, muscle tissue, and feather samples were collected from wild, wild-caught captive, and seized parrots across 13 countries (Table 1; Fig. 1). Samples were obtained from nestlings as part of ongoing Mauritius Parakeet management from 1993 to 2015. Rose-ringed Parakeets on Mauritius were mist-netted from 2009 to 2012. Samples from the Seychelles were obtained postmortem from Rose-ringed Parakeets in 2014 and as part of long-term Seychelles Black Parrot monitoring from 2009 to 2012. Further samples obtained from 2013 to 2016 from wild populations of Rose-ringed Parakeets in the United Kingdom (Kent), Germany, Senegal, Nigeria, South Africa, Japan, Pakistan, and Bangladesh were screened for BFDV where possible at the Durrell Institute of Conservation and Ecology (DICE) (University of Kent, United Kingdom) as part of a separate whole-genome sequencing project. Under the same project, samples were obtained from subadult (<3 years) captive Rose-ringed Parakeets collected from nests in Gambia in 2014 and from wild Grey-headed Parakeets in Vietnam in 2015. Samples were also obtained from an illegal shipment of parrots seized in 2015, including Timneh Parrots, thought to have originated in Ivory Coast, and from Rose-ringed Parakeets, thought to have originated in Senegal. Samples were collected postmortem from 2 Rose-ringed Parakeets in 2012 and 2013 from the United Kingdom (Greater London). One of these birds had plumage abnormalities characteristic of PBFD, and disease was confirmed through histopathological examination. The second bird had normal plumage. Samples from both cases were screened with a real-time polymerase chain reaction (PCR) assay, and both were BFDV positive (Sa et al. 2014). Samples from these cases were subsequently sent to DICE for viral characterization.

This research was conducted under the University of Kent ethical guidelines (0018-DF-16). Sampling was undertaken in collaboration with local wildlife authorities, conservation nongovernmental and research organizations, and samples were imported to the United Kingdom under the following license numbers: TARP/2015/052, TARP/2013/210, TARP/2015/213, TARP/2015/243, TARP/2015/212, TARP/2015/055, ITIMP17.0656, TARP/2013/307, TARP/2015/228, TARP/2012/292, TARP/2016/105, TARP/2013/182, and TARP/2015/085A.

DNA Extraction and Screening

An ammonium acetate DNA extraction method was used to extract bird and viral DNA prior to BFDV screening (Bruford et al. 1998). Samples were extracted in batches specific to geographic origin to reduce the risk of

Table 1. Parrot host species, country or region of origin, sample type used, and Genbank accession numbers of samples screened for Beak and feather disease virus (BFDV).

Country or region	*Sampling location*	*Species*	*Common name*	*Native or invasive*	*Wild or captive*	*No. of individuals tested*	*Positive for BFDV (%) (95% CI)*	*Sample tissue*	*Sampling year*	*Accession no.*
Bangladesh	26.270869; 88.595175	*Psittacula krameri*	Rose-ringed Parakeet	native	wild	29	100 (88.3–100)	blood	2013	KT725792–95; KX641203–27
The Gambia	13.6666; 15.05	*P. krameri*	Rose-ringed Parakeet	native	captive	3	100 (43.9–100)	blood	2014	KT725790
Germany	49.39381; –8.6952	*P. krameri*	Rose-ringed Parakeet	invasive	wild	20	0 (0–16.1)	blood	2007–2010	–
Japan	35.689488; 139.69171	*P. krameri*	Rose-ringed Parakeet	invasive	wild	15	6.7 (1.2–29.8)	feather	2015	–
Mauritius	–20.36937; 57.40602	*P. krameri*	Rose-ringed Parakeet	invasive	wild	31	16.1 (7.1–32.6)	blood	2009–2011	KT753489–93
	–20.38473; 57.44451	*P. eques*	Mauritius Parakeet	native	wild	894	26.1 (23.3–29.0)	blood	1994–2016	KT753401–88, KT753494–526; KX641202; KX641228–32
Nigeria	9.92849; 8.89212	*P. krameri*	Rose-ringed Parakeet	native	wild	11	9.1 (1.6–37.7)	blood	2014	–
Pakistan	33.242722; 73.225929	*P. krameri*	Rose-ringed Parakeet	native	wild	14	71.4 (45.4–88.3)	blood	2014	KT725800–03; KX641233–39
Senegal	14.6937; –17.44406	*P. krameri*	Rose-ringed Parakeet	native	wild	10	50 (23.7–76.3)	blood	2014	KT725796–99
Seychelles	–04.6300222; 55.4568139	*P. krameri*	Rose-ringed Parakeet	invasive	wild	23	47.8 (29.2–67.0)	muscle[a]	2014	KU888682–83, MF669120–23, MF681683
	–04.330056; 55.73839	*Coracopsis barklyi*	Black Parrot	native	wild	24	0 (0–13.8)	blood	2009–2012	–
South Africa	–26.12346; –28.00836	*P. krameri*	Rose-ringed Parakeet	invasive	wild	4	0 (0–49.0)	feather	2015	–
United Kingdom	51.4352361; 00.3325417	*P. krameri*	Rose-ringed Parakeet	invasive	wild	6[b]/2[c]	0 (0–39.0)[b]/na[c]	feather[b]/ feather follicle[c]	2013–2015	KT725791, KU888693
Vietnam	19.0636028; 104.7520944	*Psittacula finschii*	Grey-headed Parakeet	native	wild	6	66.7 (30.0–90.3)	Blood	2015	KU888690–93
Western Africa[d]		*P. krameri*	Rose-ringed Parakeet	native	captive	5	20 (3.6–62.5)	blood	2015	KU888684
		Psittacus timneh	Timneh Parrot	native	captive	8	62.5 (30.6–86.3)	blood	2015	KU888685–89

[a]*Samples obtained post-mortem.*
[b]*Samples obtained from live birds in Kent, UK.*
[c]*Nonrandom samples obtained postmortem from Psittacine beak and feather disease diagnosed parakeets in Greater London, UK.*
[d]*Samples obtained from parrots seized by trade authorities.*

Figure 1. Sampling locations of parrot species screened for Beak and feather disease virus *(BFDV) and the number of BFDV positive individuals in each study location.*

contamination between samples from different regions. For blood, approximately 50–100 μL of whole blood was used from each sample and digested in 250 μL of DIGSOL lysis buffer with 10 μL of 10 mg/mL proteinase K. For skin and muscle tissue, approximately 4 mm^2 of tissue was used from each sample and digested in 250 μL of DIGSOL lysis buffer with 20 μL of 10 mg/mL proteinase K. For feather extractions, feather barbs were removed and the calamus was chopped finely prior to digestion in 250 μL of DIGSOL lysis buffer with 40 μL of 10 mg/mL proteinase K and 70 μL of 1M dithiothreitol. Extractions were quantified using a Qubit dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA) and standardized to approximately 25 ng/μL prior to BFDV screening where possible because of high yields. The only exception to this protocol was one of the U.K. Rose-ringed Parakeet samples, from which DNA was extracted prior to its being sent to DICE for analysis.

We used BFDV-specific primers to determine presence of viral DNA within the host. Screening was carried out through PCR assays targeting a 717-bp region of *rep* (Ypelaar et al. 1999). The DNA from a BFDV-infected Mauritius Parakeet was included as a positive control (Kundu et al. 2012). Reactions comprised 1 μL of extracted DNA template, 5 μL MyTaq HS Red Mix (Bioline, London), and 0.2 μL each of the forward and reverse primers at 10 pmol/μL and were made up to 10 μL with double-distilled water. The PCR annealing temperature was 60 °C for 30 cycles, and products were visualized on a 1.5% agarose gel. A negative control of molecular-grade water was included in each PCR batch. All positive PCR products were sent to Macrogen Europe (Amsterdam) for sequencing. The single samples from Rose-ringed Parakeets that tested positive for BFDV from Japan and Nigeria (Table 1) did not yield sequences of sufficient quality for further analysis. Population-prevalence estimates based on sample size were calculated. These estimates included a 0.9 test-sensitivity assumption that we derived with Epitools (Sergeant 2018).

BFDV Phylogeny

We used GENEIOUS version 8.1.7 (Kearse et al. 2012) to align and edit the DNA sequences from this study with all *rep* gene sequences available in GenBank (downloaded 29 July 2016) for phylogenetic comparison and analysis (Supporting Information). This global *rep* alignment was used to infer the best-fit substitution model with JModelTest version 2.1.7 (Posada 2008). We constructed a maximum likelihood (ML) phylogenetic tree with RAxML version 8 (Stamatakis 2014), which applies a gamma substitution model and a rapid bootstrapping heuristic procedure (Stamatakis et al. 2008). We collapsed branches with <50% bootstrap support in TreeGraph 2 (Stöver & Müller 2010) and edited and annotated the final tree in FigTree version 1.4.2 (Rambaut 2009).

Results

All individuals screened for BFDV from Bangladesh (95% CI 88.3–100%) and The Gambia (95% CI 43.9–100) were

infected (Table 1). The virus was not detected in endemic Black Parrots in the Seychelles (95% CI 0–13.8%) or in Rose-ringed Parakeet populations in Germany (95% CI 0–16.1%), South Africa (95% CI 0–49.0%), or in Kent, U.K. (95% CI 0–39.0%), despite being present in the adjoining Greater London Area. We detected BFDV in both the native (26.1%, 95% CI 23.3–29.0) and invasive parakeet (16.1%, 95% CI 7.1–32.6) species in Mauritius. We detected BFDV in Rose-ringed Parakeet samples from Pakistan (71.4%, 95% CI 45.4–88.3), Japan (6.7%, 95% CI 1.2–29.8), Nigeria (9.1%, 95% CI 1.6–37.7), and Senegal (50%, 95% CI 23.7–76.3) and in individuals seized from trade in western Africa (20%, 95% CI 3.6–62.5). Grey-headed Parakeets from Vietnam (66.7%, 95% CI 30.0–90.3) and Timneh Parrots seized in western Africa (62.5%, 95% CI 30.6–86.3) were also positive for BFDV.

BFDV in Western Africa

The ML phylogeny (Fig. 2) showed possible multiple introductions of BFDV to western Africa. Viral variants isolated from wild Rose-ringed Parakeets in Senegal formed a monophyletic clade with the single positive individual seized from illegal trade in western Africa. In contrast, the sequences isolated from Timneh Parrots confiscated during the same seizure incident and housed in an adjacent enclosure to the Rose-ringed Parakeets were more closely related to those identified in a captive African Grey Parrot and Blue-and-yellow Macaw from Taiwan (Fig. 2 & Supporting Information). Isolates from wild Rose-ringed Parakeets from southern Asia and the captive wild-caught individual from The Gambia were found to be closely related (Fig. 2; Table 1).

BFDV on the Indian Ocean Islands and in the United Kingdom

Isolates from Rose-ringed Parakeets on the Seychelles and those in introduced Rose-ringed Parakeets in Greater London were the most closely related (Fig. 2). These sequences were distantly related to the 2 isolates available from captive parrots from the United Kingdom, which instead clustered into a diverse clade of isolates obtained from captive hosts across Europe, the United States, Oceania, and southern and Southeast Asia (Fig. 2 & Supporting Information). The BFDV isolates in both native Mauritius Parakeets and invasive Rose-ringed Parakeets in Mauritius formed a monophyletic clade with little genetic variation, consistent with a single-introduction founder effect. This Mauritius clade was sister to both the clade of isolates from wild Grey-headed Parakeets in Vietnam and those obtained from wild Crimson Rosellas (*Platycercus elegans*) in Australia.

BFDV in Southern and Southeastern Asia

The majority of the isolates obtained from Rose-ringed Parakeets in their Asian native range, from both Pakistan and Bangladesh, were most closely related to one another and to the aforementioned isolate from a wild-caught captive individual from western African (Fig. 2). Conversely, the isolates obtained from Grey-headed Parakeets in Vietnam clustered into a monophyletic clade.

Wider Phylogeographical Patterns

The BFDV *rep* gene phylogenetic tree consisted of a high proportion of clades that were monophyletic by location (>70% branch support) and had founder-effect type low genetic variation, including groups of isolates from captive flocks in Thailand and a number of captive and wild host clades from Australia, Brazil, New Caledonia, and New Zealand (Fig. 2). Sequences from captive hosts in Italy, Poland, South Africa, Japan, and Australia were widely dispersed throughout the phylogeny, which suggested multiple introductions of BFDV to these countries. The distribution of BFDV isolates from captive and wild parrots in New Caledonia differed substantially, which suggested the virus in captive populations was likely introduced from European captive stocks, whereas the strain in wild populations was instead most closely related to isolates from Australia and New Zealand.

Discussion

We report the presence of BFDV in wild populations from 8 countries where the virus had not been detected previously, showing the virus is more widespread than currently recognized and may pose a risk to several threatened species. We also found the first record of BFDV in wild Rose-ringed Parakeets within their African and Asian native ranges and in Grey-headed Parakeets in southeastern Asia, invasive Rose-ringed Parakeets in the Seychelles and Japan, and wild parrots in trade within Africa. Our phylogenetic analysis revealed multiple introduction events to western Africa and close phylogenetic relationships between sequences from wild populations across geographically distinct global regions. These findings suggest the global trade in live birds and the establishment of invasive populations play a key role in the spread of infectious disease.

Conservation Implications for Infected Native Host Populations

The relationship between the spread of BFDV and the global pet trade is most evident in western Africa. Specifically, this influence can be seen in the identification of a BFDV isolate from The Gambia clustering with those originating from southern Asia and only distantly related to those isolated from neighboring Senegal. Because this isolate was detected in a wild-harvested captive individual, it is unknown whether infection occurred prior to

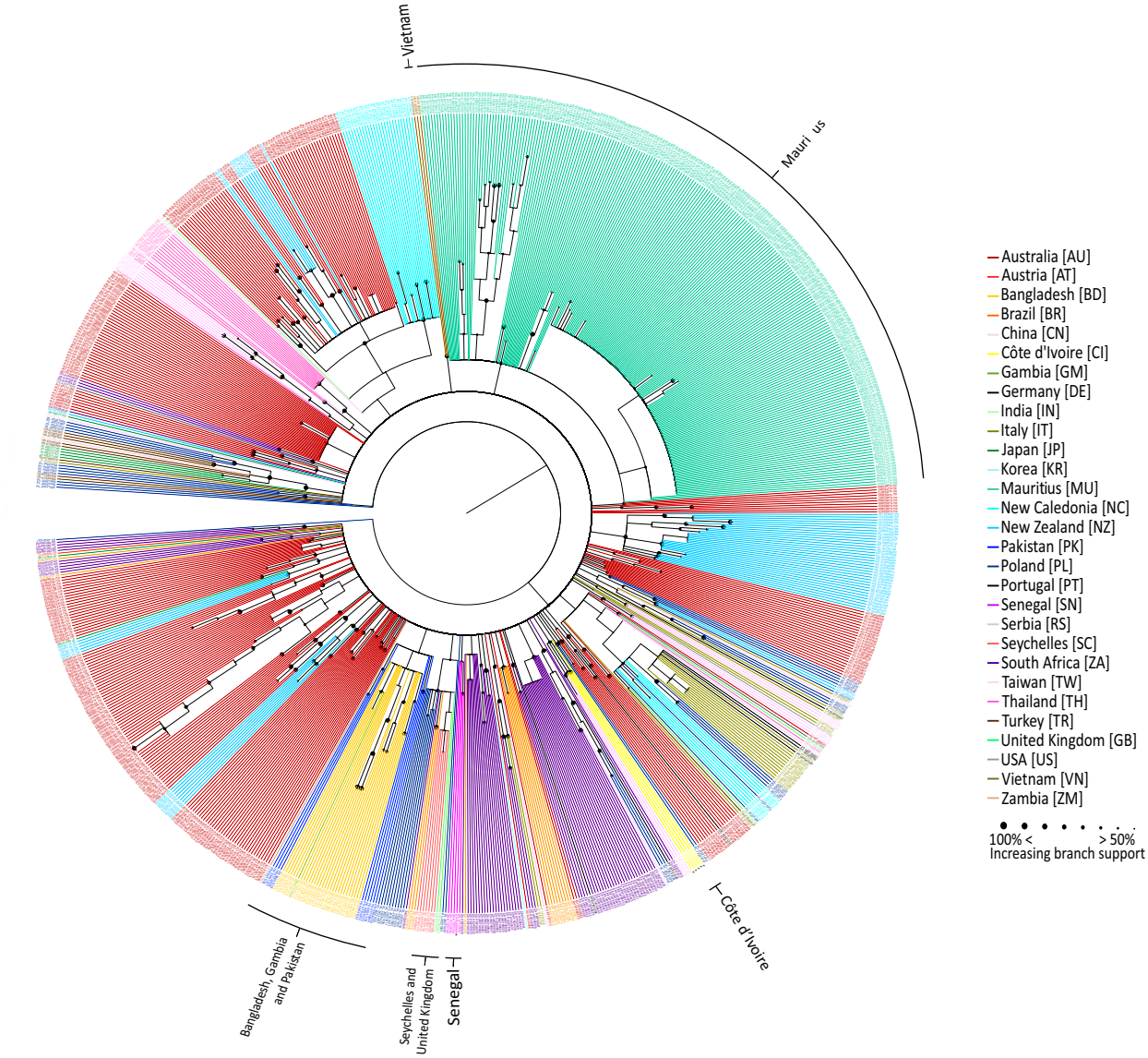

Figure 2. Maximum likelihood phylogenetic tree denoting relationships between Beak and feather disease virus *(BFDV) rep sequences. Variants sequenced for this study are highlighted and labeled, and sequences derived from birds in trade are marked with an asterisk. Branches with <50% branch support are collapsed and branch support is indicated with proportionally increasing filled circles. Branches are color coded by country of sampling as denoted in the key.*

its capture or in captivity. This finding emphasizes the need for further intensive sampling of wild parrot populations in this region as The Gambia is geographically encompassed by Senegal and the native distribution of Rose-ringed Parakeets extends through both countries (BirdLife International 2016). Therefore, these isolates would be expected to form a single clade.

The presence of markedly different BFDV strains in the Rose-ringed Parakeet and Timneh Parrots seized from illegal trafficking is noteworthy because both were housed in high-density enclosures at a single wildlife trader's holding facility. Despite their close proximity, it appears horizontal transmission did not occur and that these birds became infected with BFDV from at least 2 different sources. None of these birds showed clinical signs of disease when examined by an experienced avian veterinarian. The similarity between the isolate from the Rose-ringed Parakeet from this seizure and those from wild populations in Senegal suggests that either this individual became infected prior to capture or that wild parakeets in Senegal may have become infected by BFDV-positive parakeets that escaped captivity.

It is of conservation concern that multiple variants of BFDV occur in western Africa because this could increase

the risk of formation of novel, highly virulent strains through viral recombination (Julian et al. 2013; Jackson et al. 2015*a*). Grey and Timneh Parrots are among the most traded of all CITES-listed birds (Martin 2018*a*, 2018*b*), and increased restrictions on their international movement due to their recent listing on CITES Appendix I may help limit the spread of BFDV. However, Rose-ringed Parakeets are abundant across their native range and their population sizes are increasing (BirdLife International 2016). The confirmed presence of BFDV in these hosts highlights a risk of spill over into other sympatrically distributed species that are susceptible to PBFD (Varsani et al. 2011; Fogell et al. 2016), such as globally endangered Grey (*Psittacus erithacus*) and Timneh Parrots (BirdLife International 2017*a*, 2017*b*).

Asia has 112 parrot species, of which approximately 15% are listed on the IUCN Red List of threatened species (IUCN 2016). Over 50% of these species are declining (IUCN 2016), and little research has been conducted on the presence of BFDV in wild Asian hosts, except for a single Red Lory (*Eos bornea*) sampled from Indonesia (Sarker et al. 2013). As noted with infected species in Australia (Sarker et al. 2015*a*), Rose-ringed Parakeets in Asia appear to be endemically infected at high prevalence within a monophyletic clade, making them an abundant reservoir host. The identification of BFDV in Bangladesh and Pakistan highlights the risk of spillover into vulnerable sympatric species, such as Red-breasted Parakeets (*Psittacula alexandri*) and Blossom-headed Parakeets (*Psittacula roseata*). The identification of BFDV in Grey-headed Parakeets in Vietnam is also of conservation concern because their populations are declining due to trapping for the bird trade and widespread habitat loss, which have resulted in their being uplisted from least concern to near threatened on the IUCN Red List of Threatened Species in 2013 (BirdLife International 2017*c*).

Patterns of Viral Host Switching

The close relationship between BFDV *rep* sequences from the Seychelles and Rose-ringed Parakeets from the United Kingdom is notable because phylogenetic analysis suggests this invasive population is of southern Asian ancestry (Jackson et al. 2015*b*); therefore, it is expected that BFDV would be introduced from the same region. However, since establishment of the invasive population in 1996, there have been 5 CITES-listed imports of psittacines to the Seychelles (CITES 2016), and anecdotal reports of a feral Sulphur-crested Cockatoo (*Cacatua galerita*) on Mahé (N. Bunbury, personal communication). Any of these or imports of other non-CITES-listed parrot species into the Seychelles could have introduced BFDV, posing a high risk to the small remaining endemic population of Seychelles Black Parrots on Praslin. Both inferences that BFDV is spread through trade and that the virus displays host generality are supported by the relationship between this U.K.–Seychelles clade and the clade of isolates derived from Polish, South African, and Brazilian Old and New world parrots.

Our results suggest a single introduction of BFDV to Mauritius, and this strain is shared by the native Mauritius Parakeets and invasive Rose-ringed Parakeets. Since the introduction of BFDV to Mauritius, there has been some diversification. Isolates present in more recent samples from both parakeet populations differ from those in Mauritius Parakeets when PBFD was first observed in 1994. The Mauritius Parakeet is the last remaining of 10 Mascarene Island parrot species (Hume 2007) and has only recently recovered from a bottleneck of fewer than 20 known individuals (Duffy 1993). An outbreak of BFDV in 2005 caused the failure of a translocation attempt for further population recovery (Tollington et al. 2013) and decreased hatching success (Tollington et al. 2015). Despite the concerns of conservation managers when PBFD was first detected, Mauritius Parakeets have continued to recover. Nevertheless, as with the risk to the Seychelles Black Parrot, the pet bird trade substantially increases the likelihood of introducing novel or recombinant BFDV variants that may have higher pathogenicity than the strain currently in Mauritius.

The virus is highly prevalent in captive-breeding facilities (Julian et al. 2013), which are a large source of pet birds exported internationally and a likely source of infection worldwide (Harkins et al. 2014). The virus also has the potential to substantially affect the pet bird trade economically. For example, it was estimated that in the past commercial aviculturists in South Africa lost up to 20% of their flocks to PBFD annually (Heath et al. 2004). However, the benefits of conserving global parrot biodiversity within their native ranges and managing infectious disease within these populations extend far beyond their captive market value. Rose-ringed Parakeets have established invasive populations across Europe (Jackson et al. 2015*b*; BirdLife International 2016), and, given that captive parrots in Germany, Portugal, Spain, Italy, and Poland have tested positive for BFDV (De Kloet & De Kloet 2004; Raue et al. 2004; Julian et al. 2013), the virus is presumably also present in other European wild flocks outside the United Kingdom. Although the presence of BFDV in invasive populations across Europe poses little direct threat to wild parrot populations globally, it is valuable epidemiological data and will aid the identification of viral movement pathways and guide the development of national policies (Harkins et al. 2014).

The absence of BFDV in samples from wild Rose-ringed Parakeets in South Africa is likely due to the inadequacies of small sample sizes. Subsequent to the collection of these feather samples, clinical signs of PBFD were observed in Rose-ringed Parakeets in Randburg (C. Symes and D. Hernández Brito, personal communication). It is possible that these signs are not linked to PBFD or that the sampled feathers were grown in prior to the

establishment of novel infection in the population. The virus is already present in endemic Cape Parrots (*Poicephalus robustus*) in eastern South Africa (Regnard et al. 2014), and, although the distribution of Rose-ringed Parakeets in South Africa does not yet overlap with that of Cape Parrots, their rapid population growth may soon increase the risk of introducing a novel strain to an already-infected vulnerable endemic species. Consequently, we recommend more intensive surveillance of invasive Rose-ringed Parakeet populations in South Africa.

Value of Large-Scale BFDV Surveillance

Our results illustrate the value of disease screening samples gathered for genetic studies or over the course of long-term population monitoring. However, data sets comprising a large number of random samples are required to support the absence of infection with statistical confidence (DiGiacomo & Koepsell 1986). It should also be considered that BFDV detection is improved by using multiple sample types (e.g., Raue et al. 2004; Robino et al. 2014). Feathers typically produce low DNA yields, particularly those that have been cut off from the blood supply once fully grown (De Volo et al. 2008). Blood or muscle tissue samples, however, can produce high-quality, high-concentration DNA extracts (D.F., personal observation), but BFDV may be undetectable in the blood, whereas virions are still present in feathers or shed in feces (Hess et al. 2004). Therefore, in the case of long-term population studies, mixed sampling regimes may provide more robust assessments of global or regional infection occurrence and allow for estimates of prevalence in entire populations.

The first detection of BFDV in wild parrots native to southern and Southeast Asia and western Africa highlights the need for further research in these regions and has implications for the conservation of vulnerable sympatric species. Most of the African continent is data deficient for BFDV presence because, to our knowledge, no screening of wild populations has occurred outside southern Africa (Fogell et al. 2016). Similarly, little work has been conducted in Asia outside southeastern Asian cockatoo species. Many of our results were obtained from opportunistic samples, rather than through systematic random sampling designed to provide statistical and epidemiological confidence. As noted with Rose-ringed Parakeets in South Africa, these samples may therefore not provide a current picture of geographic occurrence of BFDV. Further screening of wild parrot populations would provide better insight into where BFDV occurs globally. This information could be used to inform conservation and management and provide a foundation for advanced studies of host immunity and susceptibility to infection.

We emphasize that dissemination of both BFDV-positive and BFDV-negative screening results are required due to the evidence that some species, such as Cockatiels (*Nymphicus hollandicus*), may be less susceptible to infection (Shearer et al. 2008). It should also be considered that the presence of infection is not always reflected in clinical signs of disease (McCallum & Dobson 2008). Therefore, once infection within a wild population is detected, the clinical signs and severity of PBFD should be noted because they differ among species. For example, diseased Mauritius Parakeets do not present with beak deformities (D.F., personal observation). Despite the more thoroughly documented presence of BFDV in threatened wild native parrot populations in South Africa (Regnard et al. 2014), Mauritius (Kundu et al. 2012), New Zealand (Jackson et al. 2015*a*), and Australia (Peters et al. 2014), the interspecific variation and long-term population impacts of PBFD are still largely unknown. Conservationists therefore need to apply a precautionary principle when managing populations at risk of infection with BFDV until risks to individual populations are better assessed.

Our data provide support for a global assessment of captive-breeding activities and strict regulation of the trade and import of parrots (Jackson et al. 2015*b*). We suggest decisions concerning the movements of parrots should include an analysis of disease risk in which probability of previous exposure or infection and the potential risk posed to wild populations are estimated. It is particularly important that these risks to biosecurity be considered in regions of high conservation importance, both for threatened parrots and other avian taxa at risk of infection (e.g., Sarker et al. 2015*b*; Amery-Gale et al. 2017). Screening for BFDV through standard and real-time PCR is quick and easy, and the evidence base for decisions will be improved with additional information on the extent of viral distribution and transmission pathways. We therefore recommend that consideration be given to the systematic screening of parrots in trade and urge conservation practitioners, parrot breeders, enforcement agencies, and others who work with threatened parrots to increase efforts to sample wild and captive parrot populations globally.

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Supporting Information

The country of origin and accession numbers for all isolates used in our phylogenetic analysis (Appendix S1) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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