TITLE: Detection of Beak and feather disease virus in native and introduced parrots and consequent implications for conservation and the pet bird trade

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 risks associated with the global pet bird trade. BFDV has been reported in several wild parrot populations, but data is lacking for many taxa and geographical areas with high parrot endemism. This data deficit impedes the development of strategies to mitigate the threats posed by BFDV. We aimed to advance understanding of BFDV distribution in many data deficient areas and determine phylogenetic and biogeographic associations of the virus from five parrot species in Africa, the Indian Ocean islands, Asia and Europe. BFDV was detected in eight countries where it was not known to occur previously, indicating the virus is more widely distributed than currently recognised. We document for the first time the presence of BFDV in wild populations of the highly traded and invasive Psittacula krameri within its native range in Asia and Africa. BFDV was detected among introduced P. krameri on the Indian Ocean islands of Mauritius and the Seychelles, raising concerns for island endemic species in the region. Examination of the phylogenetic relationships between viral sequences, including those detected among wild-sourced parrots seized from illegal trade in Western Africa, revealed likely pathways of transmission between populations. A close 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.
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 some of the most frequently traded birds listed under CITES appendices (Pain et al. 2006) and, consequently, the pet trade has driven cross-border movements of over 19 million parrots since 1975 (CITES 2016). This has exacerbated the establishment of numerous introduced populations, most notably the highly invasive rose-ringed parakeet (Psittacula krameri); with breeding populations established in over 35 countries across five 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. PBFD, first described in the 1970s (Pass & Perry 1984) in the South Pacific (Ritchie et al. 1989; Heath et al. 2004; Harkins et al. 2014), is thought to have post-Gondwanan origins due to the paucity of ancestral non-Australian clades and the infrequent observations across other regions of high parrot endemism such as Africa and South America (Raidal et al. 2015). All psittaciformes are susceptible to infection (Sarker et al. 2014), with PBFD typically characterised by chronic symmetrical feather abnormalities and 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 is thought to have been facilitated by the global trade in live parrots (e.g. Varsani et al. 2011; Harkins et al. 2014), its high environmental persistence and ability to transmit 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 five further subspecies (Fogell et al. 2016). Infection of parrots in captivity has been reported in at least 33 countries whilst the virus is known to occur 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. 2015a). 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). However, both invasive populations and captive individuals have tested positive for BFDV (Kundu et al. 2012; Julian et al. 2013; Sa et al. 2014). As a result of the rapid adaptability and successful establishment of rose-ringed parakeets globally, it has become a high-risk reservoir host and vector for BFDV, particularly where its distribution overlaps with that of vulnerable parrot species. These concerns have prompted actions such as the recent efforts of the Seychelles Islands Foundation and Seychelles government to eradicate rose-ringed parakeets on the island of Mahé as a precautionary measure to minimise threats to the endemic Seychelles black parrot (Coracopsis barklyi). This eradication campaign was launched in 2013 in response to concerns over biosecurity risks (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 our understanding of the biogeography and origins of BFDV, the potential conservation impacts of PBFD, and impedes the development of effective approaches to prevent BFDV spread to high risk areas.
Here we aimed to (i) determine the presence of BFDV in native and introduced wild parrot populations in data deficient regions and taxa across three continents, and (ii) 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. Emphasis was placed on the rose-ringed parakeet due to 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, Figure 1). Samples were obtained from nestlings as part of ongoing Mauritius parakeet species management from 1993 to 2015. Invasive rose-ringed parakeets on Mauritius were mist-netted from 2009 to 2012. Samples from the Seychelles were obtained post-mortem 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 UK, Germany, Senegal, Nigeria, South Africa, Japan, Pakistan and Bangladesh that were sent to the Durrell Institute of Conservation and Ecology (University of Kent, UK) as part of a separate whole genome sequencing project were screened for BFDV where possible. Under the same project, samples were obtained from sub-adult (<3 years) captive rose-ringed parakeets known to have been collected from the nest in Gambia in 2014, and wild grey-headed parakeets in Vietnam in 2015. Samples were obtained from an illegal shipment of parrots seized in 2015, including Timneh parrots, thought to have originated in Ivory Coast, and rose-ringed parakeets, thought to have originated in Senegal. Samples were collected post-mortem from two rose-ringed parakeets in 2012.
and 2013 from the UK. One of these birds had plumage abnormalities characteristic of PBFD, which was confirmed through histopathological examination, whilst the second had normal plumage. Samples from both cases were screened with a real-time PCR assay and were both BFDV-positive (Sa et al. 2014). Samples from these cases were subsequently sent to DICE for viral characterisation.

This research was conducted under the University of Kent ethical guidelines (0018-DF-16). Sampling was undertaken in collaboration with local wildlife authorities, conservation NGOs and research organisations, and imported to the UK on the following licence numbers: TARP/2015/052, TARP/2013/210, TARP/2015/213, TARP/2015/243, TARP/2015/212, TARP/2015/055, TARP/2013/307, TARP/2015/228, TARP/2012/292, TARP/2016/105, TARP/2013/182, 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 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 and standardized to approximately 25 ng/µl prior to BFDV screening where possible due to high yields.

The only exception to this protocol was one of the UK rose-ringed parakeet samples, from which DNA was extracted prior to being sent to DICE for analysis.
BFDV specific primers were used to determine presence of viral DNA within the host. Screening was carried out through PCR assay targeting a 717-bp region of rep (Ypelaar et al. 1999) with DNA from a BFDV-infected Mauritius parakeet included as a positive control (Kundu et al. 2012). Reactions comprised 1 µl of extracted DNA template, 5 µl MyTaq™ HS Red Mix (Bioline, USA), 0.2 µl each of the forward and reverse primers at 10 pmol/µl and made up to 10 µl with double-distilled water. PCR annealing temperature was set to 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 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 to further analyse. Population prevalence estimates based on sample size were calculated including a 0.9 assumption of test sensitivity using Epitools (Sergeant 2018).

**BFDV phylogeny**

GENEIOUS 8.1.7 (Kearse et al. 2012) was used to align and edit all DNA sequences, together with all rep gene sequences available in GenBank (downloaded 29 July 2016) for phylogenetic comparison and analysis (Supplementary Table 1). This global rep alignment was used to infer the best fit substitution model with JModelTest 2.1.7 (Posada 2008). A Maximum Likelihood (ML) phylogenetic tree was constructed using RAxML Version 8 (Stamatakis 2014) with Gamma substitution model and rapid bootstrapping (RBS) heuristic procedure (Stamatakis et al. 2008). Branches with <50% bootstrap support were collapsed using TreeGraph 2 (Stöver & Müller 2010) and the final tree was edited and annotated in FigTree v1.4.2 (Rambaut 2009).

**RESULTS**

Screening results and population prevalence estimates are presented in Table 1. All individuals screened for BFDV from Bangladesh (95% CI 88.3–100%) and The Gambia (95% CI 43.9–100%) were found to be infected. BFDV was not detected in endemic black parrots in the Seychelles (95%
(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, UK (95% CI 0–39.0%), despite being present in the adjoining Greater London Area. As published in Kundu et al. (2012) 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 but we present here a substantially more comprehensive coverage of sampling across both species. BFDV was also detected 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%), Senegal (50%, 95% CI 23.7–76.3) and those seized from trade in Western Africa (20%, 95% CI 3.6–62.5%), as well as in grey-headed parakeets from Vietnam (66.7%, 95% CI 30.0–90.3%) and in Timneh grey parrots seized in Western Africa (62.5%, 95% CI 30.6–86.3%).

**BFDV in Western Africa**

The ML phylogeny (Figure 2) suggests that there have been multiple introductions of BFDV to Western Africa. Viral variants isolated from wild rose-ringed parakeet hosts in Senegal formed a monophyletic clade with the single positive individual seized from illegal trade. In contrast, the sequences isolated from Timneh parrots that were seized in the same instance and housed in an adjacent enclosure to the rose-ringed parakeets, are more closely related to those identified in captive African grey parrot and blue-and-yellow macaw hosts from Taiwan (Figure 2, Supplementary Table 1). An indication of likely recent intercontinental transmission is the close relationship between isolates from wild rose-ringed parakeets from Southern Asia and the captive wild-caught individual from The Gambia screened for this study (Figure 2, Table 1).

**BFDV on the Indian Ocean Islands and in the UK**

Isolates from rose-ringed parakeets on the Seychelles and those in introduced rose-ringed parakeets in Greater London, UK were the most closely related (Figure 2). These sequences were distantly related to the two isolates available from captive parrots from the UK, which instead clustered...
into a diverse clade of isolates obtained from captive hosts across Europe, the USA, Oceania, Southern and Southeast Asia (Figure 2, Supplementary Table 1). The BFDV isolates present within both the native Mauritius parakeet and invasive rose-ringed parakeet hosts on 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 South-eastern Asia**

The majority of the isolates obtained from rose-ringed parakeets within 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 (Figure 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 consists of a high proportion of clades that are monophyletic by location (>70% branch support) with founder-effect type low genetic variation; including groups of isolates obtained from captive flocks in Thailand, and a number of captive and wild host clades from Australia, Brazil, New Caledonia and New Zealand (Figure 2). Sequences from captive hosts in Italy, Poland, South Africa, Japan and Australia were widely dispersed throughout the phylogeny, suggesting multiple introductions of BFDV to these countries. The distribution of BFDV isolates from captive and wild parrots in New Caledonia differed substantially, suggesting that the virus detected in captive populations was likely introduced from European captive stocks, whilst the strain in wild populations is instead most closely related to isolates from Australia and New Zealand.

**DISCUSSION**
We report the presence of BFDV in wild populations in eight new countries, showing the virus is more widespread than currently recognised and may pose a risk to several threatened species. We also present the first record of BFDV in wild rose-ringed parakeets within their African and Asian native ranges, from grey-headed parakeets in South-eastern Asia, invasive rose-ringed parakeets in the Seychelles and Japan, as well as in 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 that both 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, the identification of a BFDV isolate from the Gambian sampled parrots clustering with those originating from Southern Asia, and only distantly related to those from neighbouring Senegal. As this isolate was detected in a wild-harvested captive individual, it is unknown whether infection occurred prior to its capture or in captivity. This finding emphasises the requirement for further intensive sampling of wild parrot populations in this region as The Gambia is geographically encompassed by Senegal, with a contiguous native distribution of rose-ringed parakeets (IUCN 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 parrot hosts seized from illegal trafficking is noteworthy as both were housed at a single wildlife trader’s holding facility in high density enclosures. Despite their close proximity, it appears horizontal transmission did not occur and that these birds became infected with BFDV from at least two different sources. Additionally, 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 escaped from captivity.

It is of conservation concern that multiple variants of BFDV occur in Western Africa; potentially increasing the risks posed by viral recombination in the formation of novel highly virulent viral strains (Julian et al. 2013; Jackson et al. 2015a). Grey and Timneh parrots are among the most traded of all CITES-listed parrots (Martin 2018a, 2018b), and increased restrictions on international movements 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, with increasing population sizes (BirdLife International 2012). The confirmed presence of BFDV within these hosts highlights a risk of spillover into other sympatrically distributed species which are susceptible to PBFD (Varsani et al. 2011; Fogell et al. 2016) such as globally Endangered grey (Psittacus erithacus) and Timneh parrots.

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 show declining population trends (IUCN 2016) and little research has been conducted on the presence of BFDV in wild Asian hosts except a single red lory (Eos bornea) sampled from Indonesia (Sarker et al. 2013). As noted with infected species in Australia (Sarker et al. 2015a), rose-ringed parakeets within 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) (IUCN 2016). The identification of BFDV in grey-headed parakeets in Vietnam is also of conservation concern as they are in population decline due to the impacts of trapping for the bird trade and widespread habitat loss, resulting in their up-listing from Least Concern to Near Threatened by the IUCN in 2013 (BirdLife International 2013).
Patterns of viral host-switching

The close relationship between BFDV rep sequences from the Seychelles and rose-ringed parakeets from the UK is notable, as phylogenetic analysis suggests that this invasive population is of Southern Asian ancestry (Jackson et al. 2015b) and it was therefore expected that BFDV would have been introduced from the same region. However, since establishment of the invasive population in 1996 there have been five CITES-listed imports of psittacines to the Seychelles (CITES 2016), and anecdotal reports of a feral sulphur-crested cockatoo (Cacatua galerita) on Mahé (Pers. Comm. N. Bunbury). 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 UK–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 parakeet hosts. Since the introduction of BFDV to Mauritius there has been some diversification, with isolates present in more recent samples from both parakeet populations differing from those in Mauritius parakeets when PBFD was first observed in 1994. The Mauritius parakeet is the last remaining of ten 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 initial concerns of conservation managers when PBFD was first detected, Mauritius parakeets have fortunately continued to recover after these setbacks. Nevertheless, as with the risk to the Seychelles black parrot, the pet trade substantially increases the likelihood of introducing novel or recombinant BFDV variants that may have higher pathogenicity than the strain currently in Mauritius.
BFDV 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). BFDV is already known to have a substantial economic impact on the pet trade where, for example, it was estimated that 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 (BirdLife International 2012; Jackson et al. 2015b) 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 UK. Whilst the presence of BFDV within 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, to guide the development of national policies (Harkins et al. 2014).

The absence of BFDV in samples from South African rose-ringed parakeets is likely due to the inadequacies of small sample sizes as, subsequent to the collection of these feather samples, clinical signs of PBFD were observed in rose-ringed parakeets in Randburg (Pers. Comm. C. Symes and D. Hernández Brito). 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 within the population. BFDV is already present within endemic Cape parrot (Poicephalus robustus) populations occurring in the east of the country (Regnard et al. 2014) and, whilst the distribution of rose-ringed parakeets within 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 would recommend more intensive surveillance of invasive rose-ringed parakeet populations in South Africa.
Value of large-scale BFDV surveillance

This study illustrates the value of screening samples gathered for genetic studies or over the course of long-term population monitoring. However, datasets 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 good quality, high concentration DNA extracts (D. Fogell Pers. Obs.), but BFDV may be undetectable in the blood whilst virions are still present in feathers or shed in faeces (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 also allow for whole population prevalence estimates.

The first detection of BFDV in wild parrot species native to Southern and South-eastern Asia, as well as 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 as, to our knowledge, no screening of wild populations has previously occurred outside of Southern Africa (Fogell et al. 2016). Similarly, little work has been conducted in Asia outside South-eastern Asian cockatoo species. Many of the results presented here were obtained from opportunistic samples, rather than systematic studies fulfilling random sampling assumptions for the purposes of 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 is present globally for conservation and management purposes, as well as a foundation for advanced studies into host immunity and susceptibility to infection.
We emphasise that dissemination of both BFDV-positive and -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 since this varies between species (e.g. diseased Mauritius parakeets do not present with beak deformities; Pers. Obs. D. Fogell). 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. 2015a) 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 from infection with BFDV until risks to individual populations are better assessed.

Our data provide support for recommendations to assess global captive-breeding activities and to strictly regulate the trade and import of parrots (Jackson et al. 2015b), particularly to regions with high parrot endemism or vulnerable taxa. Improved management of transmission through trade may also be valuable to the health of other non-parrot avian taxa at risk of infection as BFDV has been found to host-switch (Sarker et al. 2015b; Amery-Gale et al. 2017). Management strategies should include a disease risk analysis, evaluating whether previous exposure or infection with BFDV could have occurred and, consequently, whether this poses a risk to other individuals or species. BFDV screening through standard and real-time PCR is quick and easy, so consideration should be given to systematic screening of all parrots in legal trade. Our research confirms that BFDV is more widespread than previously known and requires further investigation into when it was introduced to these populations, as well as whether this influences the current phylogeographic theory of post-Gondwanan emergence in Australia (Raidal et al. 2015). Sampling effort in wild and captive parrot populations should be increased globally to better assess the extent of viral distribution and transmission pathways.
LITERATURE CITED


De Kloet E, De Kloet SR. 2004. Analysis of the beak and feather disease viral genome indicates the existence of several genotypes which have a complex psittacine host specificity. Archives of Virology 149:2393–2412.


Table 1 Host species, country or region of origin, sample type used and Genbank accession numbers of samples screened for BFDV in this study.

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<th>Country / Region</th>
<th>Sampling location</th>
<th>Species</th>
<th>Common Name</th>
<th>Native / Invasive</th>
<th>Wild / Captive</th>
<th>Tested</th>
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<th>Tissue</th>
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<td></td>
</tr>
<tr>
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<td>Psittacula</td>
<td>Rose-ringed</td>
<td>Native</td>
<td>Wild</td>
<td>10</td>
<td>50 (23.7 – 76.3)</td>
<td>Blood</td>
<td>2014</td>
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</tr>
<tr>
<td></td>
<td>-04.330056; 55.73839</td>
<td></td>
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<td>Black parrot</td>
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<td>Wild</td>
<td>24</td>
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<td>Blood</td>
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<td></td>
</tr>
<tr>
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<td>Species</td>
<td>Status</td>
<td>Environment</td>
<td>Genotype</td>
<td>GenBank Accessions</td>
<td>Year</td>
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<td>Invasive</td>
<td>Wild</td>
<td>4</td>
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<td>Feather</td>
<td>2015</td>
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<td>Wild</td>
<td>6(^b/2)</td>
<td>0 (0 – 39.0)(^b/)</td>
<td>Feather(^b/)</td>
<td>2013 - 2015</td>
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<td>Wild</td>
<td>6</td>
<td>66.7 (30.0 – 90.3)</td>
<td>Blood</td>
<td>2015</td>
<td>KU888690 - 93</td>
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<td>Native</td>
<td>Captive</td>
<td>5</td>
<td>20 (3.6 – 62.5)</td>
<td>Blood</td>
<td>2015</td>
<td>KU888684</td>
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<td>Psittacus timneh</td>
<td>Timneh</td>
<td>Native</td>
<td>Captive</td>
<td>8</td>
<td>62.5 (30.6 – 86.3)</td>
<td>Blood</td>
<td>2015</td>
<td>KU888685 - 89</td>
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*Samples obtained from captive seized parrots; \(^a\) samples obtained post-mortem, \(^b\) samples obtained from live birds in Kent, UK; \(^c\) non-random samples obtained post-mortem from PBFD diagnosed parakeets in Greater London, UK.
Figure legends

**Figure 1** Sampling locations of the species screened for BFDV and the number of BFDV positive individuals in each location described in this study.

**Figure 2** Maximum Likelihood phylogenetic tree denoting relationships between global BFDV rep sequences, where variants sequenced for this study have been highlighted and sequences derived from birds in trade have been marked with an *. Branches with <50% branch support have been collapsed and branch support is indicated with proportionally increasing filled circles. Branches are coloured based on country of sampling as denoted in the key.