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Genetic diversity of the Rep gene of beak and feather disease virus in South Africa

Brief Report

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Summary. A study on the genetic variation of *Beak and feather disease virus* (BFDV) isolates in South Africa was performed by amplifying and sequencing a region within the ORF1 of the genome. Six different BFDV isolates were found in 15 psittacine species from 6 regions within South Africa, representing three unique isolates and three isolates that clustered into a budgerigar lineage (BG) previously described.

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Psittacine beak and feather disease (PBFD) is known to affect over 40 species of wild and captive Old and New World psittacines and is characterized by the roughly symmetrical loss of feathers and feather abnormalities. Birds may also suffer from beak and claw deformities, eventually dying as a result of immunosuppression [5]. Beak and feather disease virus (BFDV), the causative agent of PBFD, belongs to the genus *Circovirus* in the family *Circoviridae* and is between 14–17 nm in diameter, of icosahedral morphology and non-enveloped [6]. It has a circular, ss-DNA genome <2 kb in size in an ambisense organisation. Genetic variation in the Rep (ORF1) and coat protein (ORF2) genes is evident from recent studies on Australian, New Zealand and African isolates [2–4, 7]. The level of genetic diversity in southern African BFDV is similar to that in Australia and New Zealand and separate into 8 lineages, 3 being unique to the region [3].

This paper describes the high degree of sequence conservation in the Rep gene of South African isolates from a large number of psittacine species and a wide geographic region, comparable to known BFDV isolates.

Blood samples were obtained from a total of 161 captive psittacine birds bred by aviculturalists from different regions in South Africa, representing 19 psittacine

species of various origins (Table 1). DNA was extracted [QIAamp DNA Mini Kit (QIAGEN)] according to the manufacturer's protocol. A region within ORF1 was amplified by PCR using the primer set PB F1 (5′-AACCCTACAGACGGC GAG-3′) and PB R1 (5′-GTCACAGTCCTCCTTGTACC-3′) and/or PB F2 (5′-AACCATGCGGTCCAAGGA-3′) and PB R2 (5′-TATCAGTAATTGATGGG GTGGG-3′), designed in a previous study [1]. The amplified products were identified by electrophoresis on a 0.8% agarose gel containing ethidium bromide. All PCR products positive for BFDV were digested with *Hae*III at 37 °C for 3 hrs. Restriction fragments were separated on a 2% agarose gel containing ethidium bromide and compared to the RFLP profiles (RFLP I, II, III, IV and V) [1].

PCR products of isolates from two ring-necked parakeets [*Psittacula krameri*] and from a jardine parrot [*Poicephalus gulielmi massaicus*] representing RFLP profiles I, II and VI, respectively (Table 1) were ligated to pGEM^{TM}-T Easy Vector (PROMEGA). The resulting plasmids were used to transform competent *Escherichia coli* JM109 cells, the presence of inserts was confirmed by digestion with *Eco*R1, and the products were separated on a 0.8% agarose gel containing ethidium bromide. Three additional isolates from budgerigars [*Melopsittacus undulatus*] representing previously observed RFLP profiles III, IV and V [Table 1] that were cloned into pGEM^{TM}-T Easy Vector (PROMEGA) in a previous

Table 1. Table indicating the psittacine species tested, source and RFLP profile observed

Psittacine species	Common name	Species origin	Source[a]	RFLP (bp)[b]
Psittacula krameri	Ring necked parakeet	India	FS, WC	I, II
Psittacula roseata	Blossom headed parakeet	India	FS	–
Poicephalus rueppellii	Ruppel	Africa	FS	I
Melopsittacus undulatus	Budgerigar	Australia	EC, G, KZN, FS	I, III, IV, V
Psittacus erithacus	African grey parrot	Africa	FS, KZN	I
Aratinga sp.	Conure	South America	FS, KZN	I, VI
Poicephalus senegalis	Senegal parrot	Africa	FS, G	I
Amazona sp.	Amazon	South America	FS, G	I
Eclectus roratus	Eclectus	Australia	FS, KZN	I
Poicephalus gulielmi massaicus	Jardine	Africa	G, KZN	I, VI
Pollytelis alexandrae	Princess of Wales	Australia	FS	–
Psittacula alexandri alexandri	Moustache	South East Asia	FS	–
Neotis denhami	Stanley	South East Asia	FS	I
Trichoglossus sp.	Lorikeet	Australia	FS	I
Poicephalus rufiventris	Red bellied parrot	Africa	KZN	I
Poicephalus robustus	Cape parrot	Africa	KZN	I, VI
Agapornis roseicollis	Lovebird	Africa	LP, KZN	I
Poicephalus cryptoxanthus	Brown headed parrot	Africa	FS	–
Poicephalus sp.	Grey headed parrot	Africa	FS	I

[a]Provinces in South Africa: *EC* Eastern Cape province, *FS* Free State province, *G* Gauteng province, *KZN* KwaZulu Natal province, *LP* Limpopo province and *WC* Western Cape province

[b]RFLP (bp) where: I-450, 250 and ∼50, II-460, 250 and ∼50, III-410, 225 and 50, IV-425, 270 and 50, V-425, 225 and 50 and VI-425, 250, 50 and <50

Table 2. Beak and feather disease virus reference sequences used in this study

Host species	Common name	Isolate	Origin	GenBank Accession no.
Psephotus haematogaster	Bluebonnet	BB-WA	Australia	AF311295
Agapornis roseicollis	Rosy-faced lovebird	LB-WA	Australia	AF311296
Cacatua tenuirostris	Eastern long-billed corella	ELBC-SA	Australia	AF311297
Eolophus roseicapillus	Galah	Galah-WA	Australia	AF311298
Trichoglossus haematodus	Rainbow lorikeet	LK-VIC	Australia	AF311299
Cacatua leabeateri	Major Mitchell's cockatoo	MMC-WA	Australia	AF311300
Cacatua galerita	Sulphur-crested cockatoo	SCC-NT	Australia	AF311301
C. galerita	Sulphur-crested cockatoo	SCC1-WA	Australia	AF311302
C. galerita	Sulphur-crested cockatoo	BFDV-AUS	Australia	AF080560
C. galerita	Sulphur-crested cockatoo	SCC2-NZ	New Zealand	AY148286
C. galerita	Sulphur-crested cockatoo	SCC3-NZ	New Zealand	AY148287
C. tenirostitus	Longbill corella	LC1-NZ	New Zealand	AY148289
C. galerita	Sulphur-crested cockatoo	SCC5-NZ	New Zealand	AY148290
Lorius chlorocercus	Yellow-bib lorikeet	YBL1-NZ	New Zealand	AY148292
T. haematodus	Rainbow lorikeet	RL5-NZ	New Zealand	AY148293
T. haematodus	Rainbow lorikeet	RL2-NZ	New Zealand	AY148294
Eos reticulata	Blue-streak lorikeet	BSL1-NZ	New Zealand	AY148296
E. reticulata	Blue-streak lorikeet	BSL2-NZ	New Zealand	AY148297
Psitteuteles goldei	Goldie's lorikeet	GL-NZ	New Zealand	AY148298
T. haematodus	Rainbow lorikeet	RL6-NZ	New Zealand	AY148300
Melopsittacus undulatus	Budgerigar	BG3-NZ	New Zealand	AY148301
Pionites leucogaster	White-bellied caique	WBC1-ZA	South Africa	AY450434
Psittacus erithacus	African grey parrot	AFG4-ZA	South Africa	AY450435
C. alba	White cockatoo	UC1-ZA	South Africa	AY450436
Poicephalus robustus	Cape parrot	CPA8-ZA	South Africa	AY450437
Poicephalus robustus	Cape parrot	CPA7-ZA	South Africa	AY450438
Poicephalus rueppellii	Rüppell's parrot	RP1-ZA	South Africa	AY450439
Poicephalus rufiventris	African red-bellied parrot	ARB4-ZA	South Africa	AY450440
Poicephalus gulielmi massaicus	Jardine parrot	GJP1-ZA	South Africa	AY450441
Agapornis nigrigensis	Black-cheeked lovebird	BCL1-ZAM	South Africa	AY450442
Psittacus erithacus	African grey parrot	AFG3-ZA	South Africa	AY450443
Agapornis roseicollis	Rosy-faced lovebird	AR02-1UK	United Kingdom	AY521235
Psittacus erithacus	African grey parrot	PEG07-1GE	Germany	AY521237
Psittacus erithacus	African grey parrot	PEP01-1POR	Portugal	AY521236
Psittacula krameri	Ring necked parakeet	PK1-01TX	Texas, USA	AY521234
Psittacula krameri	Ring necked parakeet	UFS 1	South Africa	DQ384622
Psittacula krameri	Ring necked parakeet	UFS 2	South Africa	DQ384621
Melopsittacus undulatus	Budgerigar	UFS 3	South Africa	DQ384623
Melopsittacus undulatus	Budgerigar	UFS 4	South Africa	DQ384624
Melopsittacus undulatus	Budgerigar	UFS 5	South Africa	DQ384625
Poicephalus gulielmi massaicus	Jardine parrot	UFS 6	South Africa	DQ384626

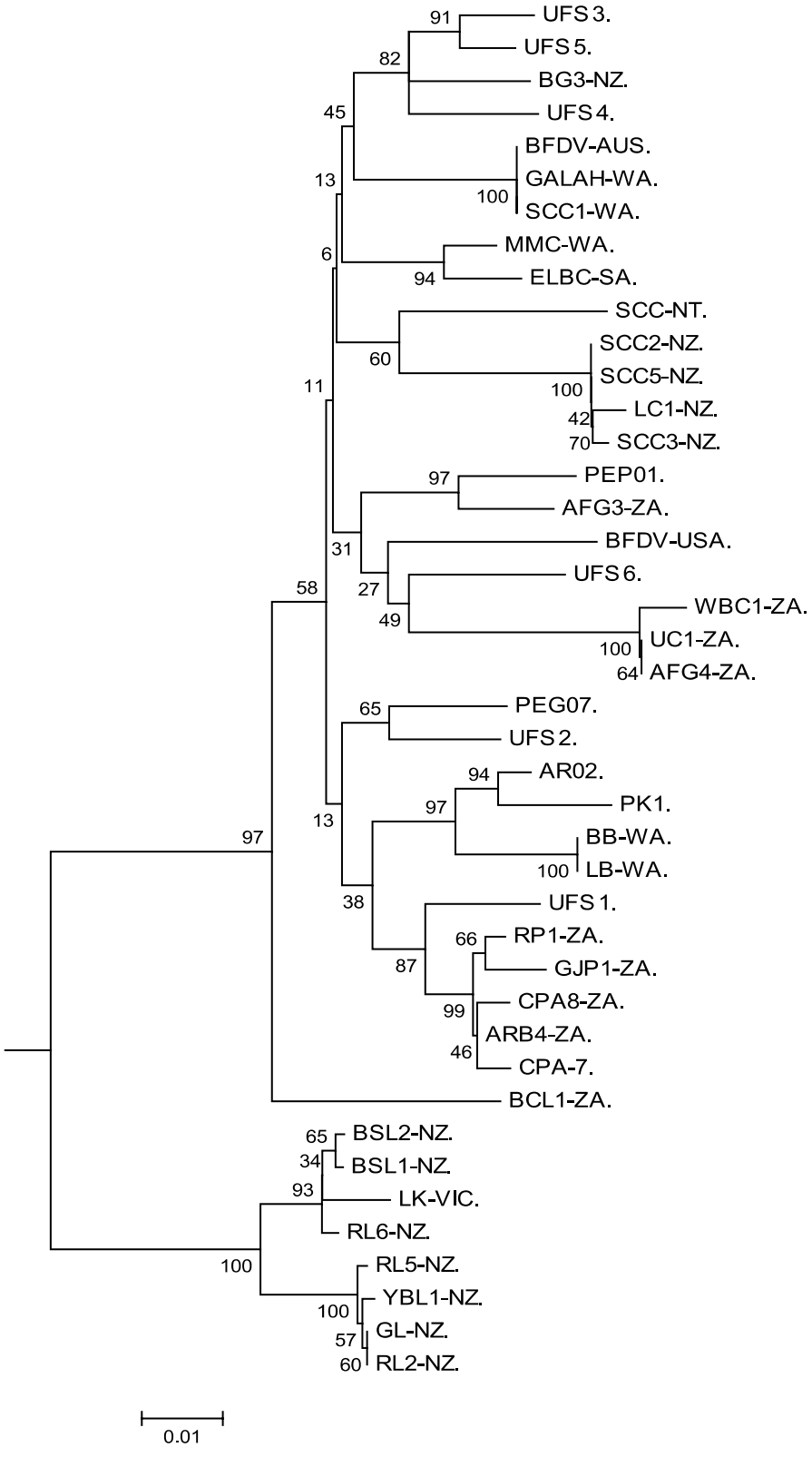
study [1] were included in the present study. Sequencing of the 6 cloned products was performed and the sequences were used in phylogenetic reconstruction with known BFDV isolates (Table 2).

The total number of blood samples (161) tested for BFDV infection, by PCR yielded 75 positive samples in 15 different psittacine species. A PCR product of approximately 700 or 870 bp represented a positive result for BFDV infection and when digested with *Hae*III, yielded one of five RFLP profiles previously described [1]. A sixth RFLP profile (RFLP VI), consisting of fragments approximately 425 bp, 250 bp, 50 bp and <50 bp, was identified in the present study. RFLP I was observed in all 15 psittacine species from different regions in South Africa that tested positive for PBFD, while RFLP III, IV and V were restricted to budgerigars [*M. undulatus*] (Table 1). RFLP II was only observed in a ring-necked parakeet [*Psittacula krameri*], while RFLP VI was restricted to a single aviary in the KwaZulu Natal province (KZN). Blood samples submitted from the same species exhibiting RFLP VI in KZN but from different bird breeders in the same region revealed RFLP I (Table 1).

Alignment of the 6 nucleotide sequences obtained in the study revealed between 94–96% homology with known BFDV ORF1 sequences. Sequence alignment also revealed a high degree of similarity, with few base differences between isolates from different species and from different regions. The topology of the phylogenetic tree in the study is similar to that described in previous studies with the Rep gene, but separation into lineages is not evident. UFS 1 [DQ384622] clustered with southern African isolates identified in a previous study and representing a unique genotype in the region [3]. However, the 87% bootstrap value makes it significantly different from the known isolates (Fig. 1). UFS 2 [DQ384621, host *Psittacula krameri*] clustered with an isolate obtained from an African grey parrot [*Psittacus erithacus* (PEG07)] in Germany, which is present in a lineage identified as being infected with a unique strain of BFDV [4]. UFS 6 [DQ384626, host *Poicephalus gulielmi massaicus*] formed a cluster with three southern African isolates, WBC1-ZA, UC1-ZA and AFG4-ZA that were found to be closely related to viruses isolated in North America [3]. UFS 3, 4 and 5 [DQ384623, DQ384624, DQ384625, host *M. undulatus*] clustered together with isolate BG3-NZ, which represents the BG lineage [7]. The 82% bootstrap value significantly divides the cluster into three, differentiating UFS 4 from BG3-NZ and the cluster formed by UFS 3 and UFS 5.

BFDV isolates that exhibited RFLP I were found in all 15 psittacine species that tested positive for PBFD. The extensive geographic distribution of these isolates indicates that RFLP I is widespread over South Africa and that there is no evidence of regional adaptation or species specificity. The division of UFS 1 [DQ384622]

Fig. 1. A neighbour-joining tree depicting the phylogenetic relationship of the Rep sequences of UFS 1–6 with those of known BFDV isolates. The full length tree was rooted with three non-psittacine avian circoviruses (Canary circovirus [GenBank accession number AJ301633], Goose circovirus [GenBank accession number AJ304456] and Columbid circovirus [GenBank accession number AJ298299]). Only the subtree showing the BFDV isolates and their bootstrap values is shown


UFS 3.
UFS 5.
BG3-NZ.
UFS 4.
BFDV-AUS.
GALAH-WA.
SCC1-WA.
MMC-WA.
ELBC-SA.
SCC-NT.
SCC2-NZ.
SCC5-NZ.
LC1-NZ.
SCC3-NZ.
PEP01.
AFG3-ZA.
BFDV-USA.
UFS 6.
WBC1-ZA.
UC1-ZA.
AFG4-ZA.
PEG07.
UFS 2.
AR02.
PK1.
BB-WA.
LB-WA.
UFS 1.
RP1-ZA.
GJP1-ZA.
CPA8-ZA.
ARB4-ZA.
CPA-7.
BCL1-ZA.
BSL2-NZ.
BSL1-NZ.
LK-VIC.
RL6-NZ.
RL5-NZ.
YBL1-NZ.
GL-NZ.
RL2-NZ.
91
82
45
13
100
6
94
60
100
11
42
70
97
31
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94
97
13
97
100
38
66
87
99
46
65
34
93
100
100
57
60
0.01

from other southern African BFDV isolates (Fig. 1) suggests that it is uniquely different and could have been separately introduced into South Africa from other isolates in the same cluster. UFS 6 [DQ384626] clustered with southern African isolates WBC1-ZA, UC1-ZA and AFG4-ZA – a cluster that is closely related to viruses isolated in North America and has been suggested to have recently been introduced into South Africa [3]. The identification of its RFLP pattern VI from a geographically localised region in this study further supports the hypothesis of a recent introduction of this virus into the country because the majority of the samples studied in South Africa [3] were obtained from a site geographically close to the aviary where this RFLP profile was found. The common ancestry that UFS 3, 4, 5 [DQ384623, DQ384624, DQ384625] and BG3-NZ share and the dominance of the South African isolates in the cluster suggest an ancestral strain of African origin. The separate evolutionary paths indicate the possibility that isolate BG3-NZ spread to New Zealand, where it was identified and assigned to the BG lineage. The importance of this study lies in the larger number of psittacine species prone to infection and wider geographical area covered in the investigation. Species specificity was not observed in the study, but genotypic association with host species, reported in some studies, is speculative because BFDV isolated from similar hosts (AFG3-ZA and AFG4-ZA isolated from *Psittacus erithacus* showing clinical symptoms) do not always form the same cluster. Furthermore, the lineages described [7] do not include all BFDV isolated from the same host species (SCC1-WA does not cluster into the CK lineage), and the topology of the phylogenetic tree based on the CP gene [3] differs from that based on the Rep gene (Fig. 1).

The overall sequence variation present in the Rep gene indicates that this region is highly conserved in all known BFDV isolates including the South African isolates identified in this study (Fig. 1). The sequence similarity extends both geographically and by species as represented by the expansive range of samples used in the study.

In conclusion, the study revealed the presence of three uniquely different BFDV isolates in South Africa, with future investigation involving the identification of any antigenic differences present, using the CP gene, which could lead to the identification of novel strains. Additionally, the larger sample size used in the current study and the wider range of psittacine species represented (as compared to previous studies) demonstrate that genetic diversity occurs in the South African isolates, although they cluster with known BFDV isolates from Australia and New Zealand. While diversity is evident, the level of gene sequence conservation between them is high in ORF 1, which is significant in the application of a universal PCR as a diagnostic test for PBFD.

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