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Psittacine beak and feather disease outbreak in captive rose-ringed parakeets (*Psittacula krameri*) in the State of Ceará, northeastern Brazil[1]

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ABSTRACT.- Duarte JLC, Olinda RG, Ospina-Pinto MC, Luz IMO, Raso TF, Araújo JL. **Psittacine beak and feather disease outbreak in captive rose-ringed parakeets (*Psittacula krameri*) in the State of Ceará, northeastern Brazil**. *Pesquisa Veterinária Brasileira 46:e07807, 2026.* Graduate Program in Animal Science, Laboratório de Medicina da Conservação, Universidade Federal da Paraíba, Rodovia 12 PB-079, Cidade Universitária, Areia, PB 58397-000, Brazil. E-mail: lealjeann@gmail.com

Psittacine beak and feather disease (PBFD) affects Psittaciformes worldwide. In Brazil, PBFD is an emerging disease that has threatened exotic and native Psittaciformes in captivity; however, reports in the country are still scarce. The aim of this study was to describe a natural outbreak of PBFD in rose-ringed parakeets (*Psittacula krameri*) from breeding facilities in the state of Ceará, Northeast of Brazil. Six fledgling parakeets presented progressive feather loss and apathy and were euthanized due to a presumptive diagnosis of PBFD. Samples from the organs were collected in 10% formalin. Feathers and fragments of the organs were frozen and later tested by polymerase chain reaction (PCR) using a conserved sequence of the viral genome, followed by sequencing and phylogenetic analysis. Grossly, multifocal to coalescent apteria were observed in rectrices and covering feathers. Microscopically, multiple areas showed a disordered increase of epithelial cells in the stratum germinativum of the skin's epithelial layer, as well as epithelial and follicular necrosis with botryoid inclusion bodies. In addition, cellular necrosis was noted in the thymus and bursa. All samples tested positive for beak and feather disease virus (BFDV) and were confirmed by sequence analysis. To the authors' knowledge, this is the first report of BFDV infection in the State of Ceará. Considering the risk of *Psittacula krameri* as a potential spreader of BFDV, we emphasize the need to establish control measures to prevent viral dissemination to neotropical psittacine birds.

INDEX TERMS: PBFD, avian circovirus, parakeets, ring-necked parakeet, Brazil.

RESUMO.- [**Surto de doença do bico e das penas dos psitacídeos em periquitos-de-colar (*Psittacula krameri*) cativos no estado do Ceará, Nordeste do Brasil.**] A doença do bico e das penas dos psitacídeos (PBFD) afeta Psittaciformes em todo o mundo. No Brasil, PBFD é uma doença emergente que ameaça

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Psittaciformes exóticos e nativos em cativeiro; entretanto, os relatos no país ainda são escassos. O objetivo deste estudo foi descrever um surto natural de PBFD em periquitos-de-colar (*Psittacula krameri*) de criatórios no estado do Ceará, Nordeste do Brasil. Seis filhotes de periquitos apresentaram perda progressiva de penas e apatia, sendo eutanasiados devido o diagnóstico presuntivo de PBFD. Amostras dos órgãos foram colhidas em formalina a 10%, e penas e fragmentos dos órgãos foram congelados e posteriormente testados por PCR utilizando uma sequência conservada do genoma viral, seguida de sequenciamento e análise filogenética. Macroscopicamente, foram observados apterias multifocais a coalescentes nas penas retrizes e coberteiras. Microscopicamente, múltiplas áreas apresentaram aumento desordenado de células epiteliais no estrato germinativo da camada epidérmica da pele; assim como observou-se necrose epitelial e folicular com inclusões botrioides. Adicionalmente, notou-se necrose celular no timo e na bursa. Todas as amostras testaram positivo para o vírus da doença do bico e das penas (BFDV) e confirmado por sequenciamento. Para o conhecimento dos autores, este é o primeiro relato de infecção por BFDV no estado do Ceará. Considerando o risco de *Psittacula krameri* como potencial disseminador do BFDV, enfatizamos a necessidade de estabelecer medidas de controle para prevenir a disseminação viral para psitacídeos neotropicais.

TERMOS DE INDEXAÇÃO: PBFD, circovírus aviário, periquito, periquito-de-pescoço-anelado, brasil

INTRODUCTION

Psittacine beak and feather disease (PBFD) is an infectious and contagious disease that affects both captive and free-living Psittaciformes worldwide (Fogell et al. 2016, Shah et al. 2023). PBFD is caused by the beak and feather disease virus (BFDV), the smallest infectious DNA virus in the animal kingdom, with viral particles measuring 14–16 nm and containing a circular single-stranded DNA genome (Ritchie et al. 1989, Kloet & Kloet 2004). Recently, BFDV was classified by the International Committee on Taxonomy of Viruses (ICTV) as a member of the species *Circovirus parrot*, which belongs to the Circoviridae family and the *Circovirus* genus (Varsani et al. 2024).

In Brazil, PBFD is currently an emerging disease in captive birds, which has threatened exotic and native Psittaciformes in various types of establishments (Ghizoni & Raso 2022). Reports of BFDV infection are scarce and documented only in captive birds in the Southeast region of the country (Werther et al. 1999, Araújo et al. 2015, Ecco et al. 2022, Philadelpho et al. 2022). Specifically, in northeastern Brazil, only one peer-reviewed case of PBFD has been reported, affecting a *Melopsittacus undulatus* in the state of Rio Grande do Norte (Batista et al. 2021).

Exotic Psittaciformes, such as *Psittacula krameri* (Scopoli, 1769), are the main source of BFDV introduction into different countries and of viral transmission to native fauna (Fogell et al. 2018). In some countries, the role of *P. krameri* as a host and transmitter of BFDV is scarcely described. In Brazil, although some clinical cases have been observed by avian veterinarians, only one case of PBFD in *P. krameri* has been reported to date (Ecco et al. 2022).

The aim of this study was to describe a natural outbreak of psittacine beak and feather disease in rose-ringed parakeets (*P. krameri*) from breeding facilities in the state of Ceará, Northeast of Brazil.

MATERIALS AND METHODS

Ethical approval. Euthanasia was performed by a licensed veterinarian as a standard welfare procedure to ensure humane management and containment of potential viral dissemination in clinically compromised birds, in accordance with the guidelines established by Federal Council of Veterinary Medicine Resolution No. 1.000/2012. This resolution specifically authorizes euthanasia in cases where animals constitute a risk to native fauna, public health, or the environment (CFMV 2012). Carcasses from euthanized animals were subsequently received at the laboratory for necropsy. Samples received by the "Universidade Federal da Paraíba" (UFPB) were routinely processed in accordance with the authorization by the University Institutional Ethics on Animal Use Committee (CEUA), permit CEUA No. 8901220725.

Clinical history and necropsy. Thirteen juvenile rose-ringed parakeets (*Psittacula krameri*) of different sexes, originating from three commercial breeders in Fortaleza, Ceará, presented a clinical history of progressive feather loss, hyporexia, weight loss, and apathy. In Breeder I, seven out of 300 birds exhibited clinical signs. In Breeder II, four out of 70 birds were affected. In Breeder III, two birds showed clinical signs; however, the total number of birds housed at the facility was not reported. All affected birds originated from the same breeding facility located in Mossoró, Rio Grande do Norte, approximately 240 km from Fortaleza. The source facility continuously introduced new birds into the flock and lacked basic biosecurity measures, including quarantine and sanitary monitoring of incoming animals. In addition to rose-ringed parakeets, the facility housed several

exotic psittacine species, including Budgerigars (*Melopsittacus undulatus*), Kakarikis (*Cyanoramphus novaezelandiae*), Alexandrine parakeets (*Psittacula eupatria*), and Pacific parrotlets (*Forpus coelestis*). At the breeding facilities in Ceará, environmental sanitation was performed using a commercial disinfectant based on potassium monopersulfate. In contrast, at the source breeding facility, sanitization procedures relied on common household disinfectants. Based on the clinical suspicion and presumptive diagnosis of PBFD and its potential threat to wildlife, the licensed veterinarian responsible for the cases euthanized birds exhibiting chronic clinical signs and submitted the carcasses for necropsy. A total of six birds (four females and two males, aged between three and six months) were submitted for *post mortem* examination. A systematic external evaluation and necropsy were performed, and feather tracts exhibiting plumage abnormalities were identified. Representative tissue samples from all organs were collected and fixed in 10% formalin. The samples were routinely processed, embedded in paraffin, sectioned at 5 µm thickness, stained with hematoxylin and eosin (HE), and examined under a standard light microscope.

DNA extraction. Feathers and fragments of liver, spleen and bursa from the six birds were collected at necropsy and stored at -20 °C until molecular analyses. Subsequently, 25 mcg of tissues were eluted in sterile PBS and lysed in a tissue disruptor (Disruptor Genie®, Scientific Industries USA) for five minutes. Then, DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions.

PCR identification. The extracted DNA samples were tested by polymerase chain reaction (PCR) using a conserved sequence (*Rep* gene) of the BFDV genome, resulting in a PCR product of 717 bp (Ypelaar et al. 1999). A 25 µl reaction mixture containing 3 µl of DNA, 0.7 µM of each primer, 12.5 µl of DreamTaq Green PCR Master Mix 2X (Thermo Fisher Scientific, USA) and nuclease-free water was added to reach the final volume. Amplification reactions were carried out with an initial denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 seconds, 60 °C for 30 seconds, 72 °C for 1 min, and a final extension at 72 °C for 10 minutes. Positive and negative controls were included in all reactions. PCR products were analyzed by electrophoresis on 1.5% agarose gels stained with GelRed® (Biotium, USA) and then purified using the Wizard® SV Gel and PCR Clean-Up System kit (Promega, Madison/WI, USA) for Sanger sequencing using an ABI 3730 DNA Analyzer (Life Technologies-Applied Biosystems, Foster City, USA).

Phylogenetic analysis. Chromatograms were edited using Bioedit software. Sequences were compared with available sequences on GenBank through the basic local alignment search tool (BLAST). Alignment and phylogenetic tree construction using the maximum likelihood method based on the Tamura-Nei model with 1000 bootstraps were conducted in MEGA 11 (Kumar et al. 2018), with the three obtained sequences from the present study and available BFDV sequences from GenBank. Pigeon circovirus (GenBank accession number OR587847) was used as the outgroup.

RESULTS

Grossly, multifocal to coalescing apteria were observed in rectrices, remiges and coverts of the capital, cervical, dorsopelvic, right and left scapular, subhumeral, pectoral, sternal and abdominal tracts (Fig. 1–3). The developing feathers were short and were all enclosed within the feather sheath.

Microscopically, multifocal areas of orthokeratotic hyperkeratosis were observed in the epidermis, with multiple areas in the stratum germinativum of the epidermal layer showing a disordered increase of epithelial cells (Fig. 4). Several developing feather follicles showed a marked deposition of keratin in the epithelial layer of the follicle (hyperkeratosis), causing a thickening of the follicle (Fig. 5). Individual necrosis or groups of epithelial cells in basal and epidermal zones of developing feathers and pulp were observed, the basal layer of the feather follicle was disordered due to the presence of necrotic cells and contained cells with enlarged nuclei with large nucleoli (Fig. 6–8), often irregular and with hyperplastic areas. In random groups of cells, there was margination of nuclear chromatin by round basophilic structures of varying sizes (botryoid inclusion bodies), associated with macrophages containing a high number of inclusions in their cytoplasm (Fig. 7), mainly within the pulp and epidermal layers. In others, the nuclei were only enlarged with chromatin margination and a clear center. The feather sheath has increased in width due to the hyperkeratosis, and some contained hyperplastic epithelial cells (Fig. 7), necrotic cell debris, and cells with basophilic intracytoplasmic inclusions. In two birds, a focally extensive lymphocytic infiltrate was observed in the stratum laxum of the dermis and a slight presence of plasma cells. In the liver and spleen, focally extensive cellular necrosis was observed. Additionally, basophilic intranuclear inclusion bodies were observed in hepatocytes, randomly distributed (Fig. 6); in some cells, the inclusions displaced the chromatin toward the nuclear periphery. In the bursa of Fabricius, several follicles were necrotic with loss of the follicular structure, and in the middle of the cell debris, botryoid inclusion bodies were observed (Fig. 9).

Samples (feathers, liver, spleen and bursa) from all birds were positive for BFDV by PCR. Sequence analysis confirmed that the obtained sequences (GenBank accession numbers PZ285421 to PZ285423)

corresponded to BFDV, with a high percentage of identity (up to 100%) to other BFDV sequences of exotic psittacine birds from Brazil and from different countries and dates available in GenBank (Fig. 10).

DISCUSSION

In the present study, clinical signs of PBFD, characterized by symmetrical loss of remiges, rectrices, and coverts feathers, were observed in young rose-ringed parakeets from three aviaries. The definitive diagnosis of PBFD was obtained through the association of macroscopic, microscopic and molecular findings. Clinically, the six birds showed nonspecific signs in addition to feather loss. In fact, young birds, in the process of molting, typically exhibit an acute form of PBFD with nonspecific signs such as weight loss and lethargy (Fogell et al. 2018, Ghizoni & Raso 2022). The presentation of clinical signs of PBFD is directly influenced by the age of the bird, the route of viral exposure, the viral load and the health status of the bird at the time of virus exposure (Pass & Perry 1984, Wellehan Jr et al. 2016). Other clinical changes that may be present in birds with PBFD but were not identified in this study include deformities in the beak and/or claws, which are frequent changes in *Cacatua* spp. (Pass & Perry 1984, Ritchie et al. 1989). In Brazil, apathy and anorexia are commonly reported among birds infected with BFDV, with many cases showing no evident clinical signs (Araújo et al. 2015, Ecco et al. 2022, Philadelpho et al. 2022).

The macroscopic presentation was characterized by the loss and abnormal development of feathers in all birds, mainly the contour and flight feathers, resulting in multifocal apteria. This is similar to the macroscopic findings described in *Psittacula krameri* in Great Britain (Sa et al. 2014).

The histological lesions observed in our cases, mainly epithelial and follicular necrosis in skin with botryoid inclusion bodies, are characteristic and expected findings in birds with PBFD, similar to other reports in rose-ringed parakeets (*P. krameri*), budgerigar (*Melopsittacus undulatus*) and yellow-crested cockatoo (*Cacatua galerita*) (Pass & Perry 1984, Sánchez-Godoy et al. 2020, Kiatipattanasakul-Banlunara et al. 2022). In addition, lesions in the bursa of Fabricius are another frequent finding of PBFD, as the virus has tropism for organs of the immune system, such as the thymus and bursa of Fabricius (Pass & Perry 1984, Ghizoni & Raso 2022), causing cellular necrosis similar to what we observed. In the present cases, necrotic lesions and inclusion bodies in the liver are uncommon findings in infected birds; however, hepatocytes can also serve as a site of viral replication in some cases (Ghizoni & Raso 2022). The splenic necrosis could not be directly associated with BFDV due to the absence of viral inclusions; therefore, it should be considered a consequence of an immunosuppressive condition and lesions resulting from secondary infections (Todd 2000).

The phylogenetic tree obtained by maximum likelihood (Fig. 10) revealed that the sequences from this study (PZ285421, PZ285422 and PZ285423) formed a clade with other Brazilian isolates, such as the *P. krameri* sequence detected in 2024 (PV818105) and the *Agapornis personatus* sequence (ON502955) detected in 2014, both from Minas Gerais, Brazil, suggesting evolutionary proximity between these sequences. Furthermore, it indicates relative similarity to *P. krameri* sequences from Europe detected in 2007 in Poland (JX221017) and in 2012 in the United Kingdom (KT725791), and to a sequence also detected in Minas Gerais in 2011 (JQ649411). Phylogenetic proximity between Brazilian and European sequences emphasizes the potential role of international bird trade in the distribution of BFDV around the world. It suggests that the dissemination of the virus may be driven by anthropogenic factors, since psittacine bird species are widely traded and transported, often with limited sanitary controls. The analysis also revealed some nodes with low bootstrap support, and these must be considered when interpreting phylogenetic relationships. This may be related to the use of the partial Rep gene, a conserved region widely used in the molecular diagnosis of BFDV worldwide, that may not provide sufficient variability for phylogenetic inference. It is important to emphasize that *P. krameri* is an exotic species in the American continent, currently being widely bred in captivity and frequently found in the international bird trade. This fact is concerning, given that this species is highly susceptible to BFDV infection and is typically observed in mixed species in breeding facilities, highlighting the potential risk of transmission of BFDV to native psittacine species.

Among Psittaciformes affected by BFDV in Brazil, exotic birds are more frequently infected, including cockatoos (*Cacatua alba*), budgerigars (*Melopsittacus undulatus*) and yellow-collared lovebirds (*A. personatus*) (Werther et al. 1999, Batista et al. 2021, Ecco et al. 2022, Philadelpho et al. 2022). However, only a few epidemiological studies with native species from southeastern Brazil, such as blue-fronted amazon parrots (*Amazona aestiva*) and blue-and-yellow macaws (*Ara ararauna*), have been published (Araújo et al. 2015, Philadelpho et al. 2022).

Currently in Brazil, PBFD diagnoses have mostly been based on molecular evaluation, using samples of feces, feathers and/or blood from birds (Araújo et al. 2015, Philadelpho et al. 2022). Few studies have performed anatomopathological evaluation of birds (Werther et al. 1999, Ecco et al. 2022), highlighting the relevance of this report, which associated gross and microscopic lesions with molecular analyses.

In this study, all affected birds were juveniles (< 6 months), and the predisposition to BFDV infection is greater in young birds due to the age-dependent immune system and the less effective adaptive immune response (Raidal & Peters 2018). Furthermore, PBFD is a potentially fatal disease characterized by viral-induced damage to lymphoid tissue and immunosuppression (Todd 2000, Kuhlman & Martin 2010, Fogell et al. 2018). Despite birds being from breeders, the possibility of stressful factors related to the origin, nutritional and/or environmental management of the birds cannot be excluded. Also, it is important to highlight that, given the contagious nature of PBFD, isolation and testing of birds that have contact with positive individuals are essential for effective control of this disease, especially in environments with high densities of birds.

CONCLUSION

To the best of the authors' knowledge, this is the first report of a natural outbreak of psittacine beak and feather disease (PBFD) in rose-ringed parakeet breeding facilities in the State of Ceará. The detection of beak and feather disease virus (BFDV) in captive parakeets in aviaries in southeastern and northeastern Brazil is a result of the massive increase in captive breeding of this species in the country, due to the intensive international exotic parrot trade in the last decades. Therefore, this study highlights the risk of *Psittacula krameri* as an important potential source of BFDV to psittacine flocks in Brazil, as well as the importance of reporting a disease that has numerous anecdotal accounts of its spread but few peer-reviewed studies. This emphasizes the urgent need to establish stricter quarantine and screening tests to control the viral dissemination among exotic species, which could result in the infection of Neotropical species.

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Conflict of interest statement.- The authors declare that they have no conflicts of interest.

Credit author statement.- José L.C. Duarte: Sample processing, histological analysis, and article writing. Roberio G. Olinda: Sample processing, histological analysis and article review. Ilanio M.O. Luz: Sample separation and preparation, and epidemiological investigation. Maria C. Ospina-Pinto: PCR technique and phylogenetic analysis. Tânia F. Raso: Supervision of PCR procedures, phylogenetic analyses and article review. Jeann L. Araújo: Supervision, histological analysis and article review.

Data availability statement.- The data supporting the findings of this study are available within the article and will be made available by the authors on request.

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Figures

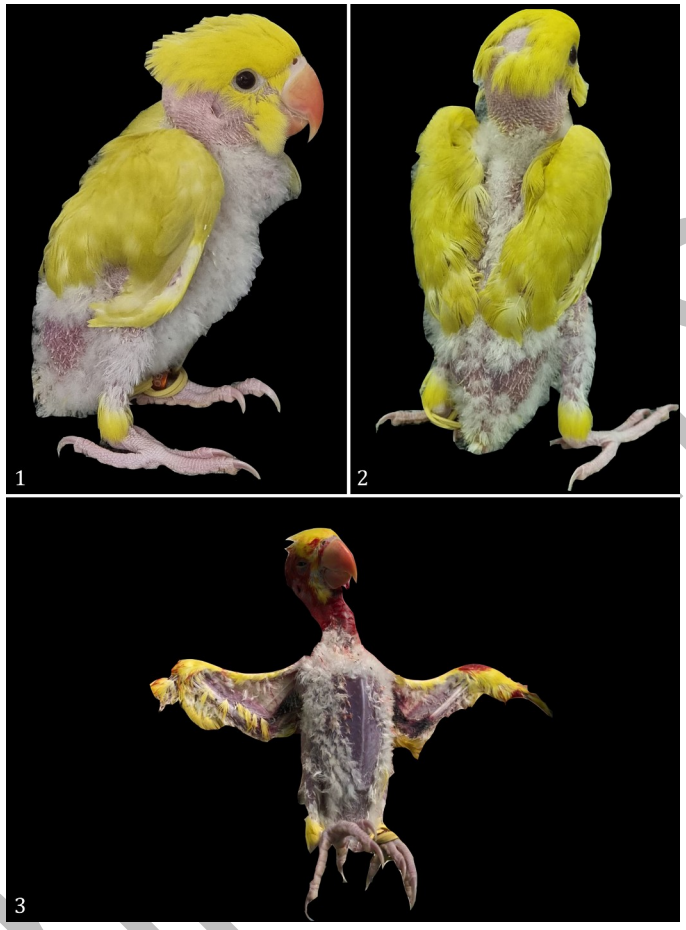

Fig. 1–3. Macroscopic plumage changes in *Psittacula krameri* with psittacine beak and feather disease (PBFD). (**1**) Apteria of the feathers of the cervical, pectoral and abdominal tracts. (**2**) Apteria of the feathers of the capital (occipital), right and left scapular and dorsopelvic tracts. (**3**) Apteria of primaries and secondaries remiges and rectrices feathers and of cervical, subhumeral, pectoral, sternal and abdominal tracts.

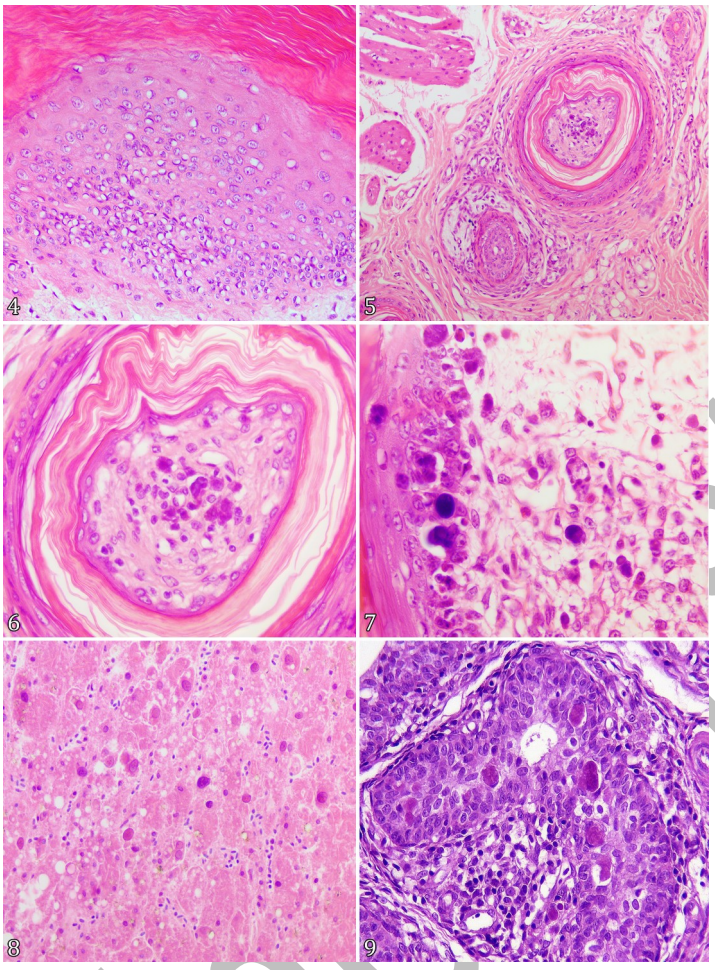

Fig. 4–9. Photomicrographs of histological changes in *Psittacula krameri* with psittacine beak and feather disease (PBFD). (**4**) Epidermis. Stratum germinativum thickened with 8 to 10 layers of epithelial cells, nuclei presenting pale spaces with peripheral chromatin. HE, obj. 40x. (**5**) Hyperkeratosis of the corneous layer of the follicular epidermis and hyperplastic epithelial cells causing a thickening of the follicle. HE, obj. 20x. (**6**) Feather pulp with epithelial cells and macrophages containing intracytoplasmic basophilic inclusions, showing loss of structural organization due to cell loss. HE, obj. 40x. (**7**) Individual necrosis in the basal layer of developing feathers with cells with enlarged nuclei, large nucleoli, intracytoplasmic basophilic inclusions and the presence of macrophages. HE, obj. 60x. (**8**) Liver with focally extensive hepatocellular necrosis and randomly distributed intranuclear inclusion bodies. In some hepatocytes, the inclusions displaced the chromatin toward the nuclear periphery. HE, obj. 40x. (**9**) Bursa of Fabricius with follicular necrosis and presence of intracytoplasmic basophilic inclusions. HE, obj. 40x.

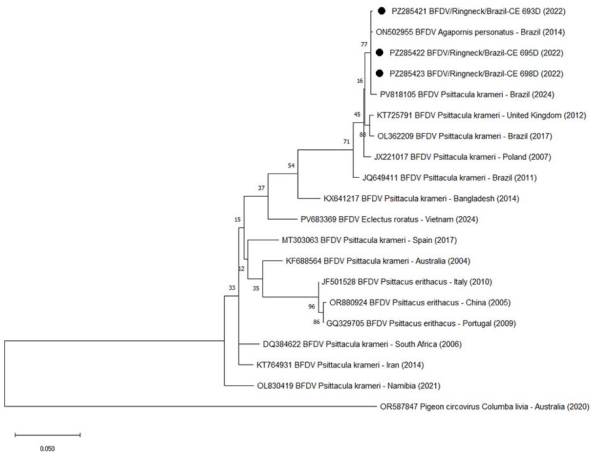

Fig. 10. Maximum likelihood phylogenetic tree constructed with beak and feather disease virus (BFDV) sequences based on the Tamura-Nei model (1,000 bootstrap replicates) in MEGA 11. GenBank accession numbers are included. Pigeon circovirus (GenBank accession number OR587847) as outgroup. Numbers indicate bootstrap support. Filled circles correspond to the BFDV sequences obtained in the present study (GenBank accession numbers PZ285421 to PZ285423).