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## PSITTACINE BIRD CITES (THE CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES OF WILD FAUNA AND FLORA) APPENDICES CLASSIFICATION, BEAK AND FEATHER DISEASE, PHYLOGENETIC ANALYSIS OF BEAK AND FEATHER DISEASE VIRUS

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#### ARTICLE INFO

## ABSTRACT

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#### Key words:

*Psittacine*, bald cocky disease, feather, open reading frames, MEGA7.

Psittacine circoviral disease affects parrots and related species, PCD commonly known as Beak and feather disease or bald cocky disease. The virus can affect birds of all ages. The virus kills feather and beak cells, and the only way to control the disease through maintaining of hygienic condition and early diagnosis. PBFD disease was first recognized in 1975 by a veterinary practitioner in Sydney. Virus spreads in a number of ways, Infection from one individual to another by direct contact, inhalation, feather dust, feeding and fecal matter. Psittacine birds that develops beak abnormalities (a longer beak, Scissors Beak and Prognathism), necrosis of the hard palate observed in severe cases. ABV has six open reading frames (ORF) with a typical 3' to 5'genome organization, the first predicted transcription unit encodes for the viral nucleoprotein (N), the second predicted transcription unit for the regulatory protein (X) and the viral phosphoprotein (P), and the third transcription unit is predicted to code for the viral matrix protein (M), the viral glycoprotein (G) and the RNA-dependent-RNA- polymerase (L). ABV X and P are encoded from overlapping primary and tertiary transcript. This study gives a proper knowledge about the early identification of Psittacine Beak and Feather Disease and implementation of action against the Psittacine Beak and Feather Disease. The evolutionary distances of the parrot bornavirus, aquatic bird borna virus and canary borna virus were computed by using the Poisson correction method and analyzed by MEGA7 software.

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## **INTRODUCTION**

Psittacine birds comes of under the order Psittaciformes which includes amazons, budgies, cockatiels. lories, lovebirds, cockatoos, caique. conures, macaw, African greys, senegals. Psittacines bird sizes range from 3.5 inches to 40 inches. Psittacines birds have a large, rounded head; short neck and legs; and usually bright plumage. Zygodactyl toes (2 point forward and 2 point back). Most live in tropical or subtropical areas and little may be found in southern temperate zones. These birds have a blunt tongue and eat seeds, nuts, fruit, buds, and other plant material. Sometimes; a few species of Psittacines birds eat insects, animals and carrion. The lories and lorikeets are specialized for feeding on floral nectar and soft fruits. Almost; all parrots nest in tree hollows and lay white eggs.

\**Corresponding author:* Yuvaraj Sampathkumar Department of Biotechnology, University of Madras, Guindy Campus, Chennai - 25 There are three families namely Loriidae - lories and Psittacidae - parrots lorikeets. and parakeets, Cacatuidae - cockatoos and cockatiels and 393 species in 92 genera within the order. The positioning of the toes is especially useful for taking hold and climbing. Psittacines birds hold their foods in one foot to eat it, hinge-like flexible beak attachment to the skull. The beak fits over the mandible. The classification of species in CITES Appendices occurs as follows: Appendix I: species at risk of global extinction, Appendix II; species that may become threatened with extinction if international trade restrictions would be absent. Nonendangered species that may be in danger because of resemblance to specimens listed in the appendices. Second-generation captive-born offspring of Appendix I Appendix III: species for which trade is species. regulated in request of certain countries.

#### Psittacine bird CITES Appendices classification



Figure 1 Borna virus morphology and physical characteristics (Courtesy :Miesle, 2016 and NCBI Reference Sequence: NC\_001607.1)

Picture 2:



Common name Scientific name EPBC status

Location

Budgerigar Melopsittacus undulates Red list: Least Concern (LC)

naturally distributed through Australia



Pionites xanthomerius Not globally threatened. CITES II Amazon River and west of the Ucayali River in Brazil, northern Bolivia, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, and Venezuela



Galarial



Cockatiel Nymphicus hollandicus Red List species status – Least Concern Australian mainland Princeton, New Jersey, USA.



Cockatoo Cacatuidae galerita CITES Appendix II Naturally occurs in Queensland, interstate and overseas



Picture 4:

Common name Scientific name EPBC status

Location

Picture 5:

Common name

Scientific name

EPBC status

Location

Picture 6:

Cockatoo-Cacatua Lophochroa leadbeateri Vulnerable in NSW and QLD, Rare in SA, Threatened in VIC.

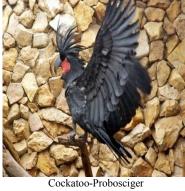
Naturally occurs in Queensland and interstate



Cockatoo-Eolophus Eolophus roseicapilla

Red List species status - Least Concern

Australian states, Cape York Peninsula. Adelaide, Perth and Melbourne, Tasmania



Cockatoo-Probosciger Probosciger aterrimus Threatened Species Australia and New Guinea



Conure-Aratinga Aratinga solstitialis Red List species status – Endangered Northeastern South America.



 Common name
 Conure-Cyanoliseus

 Scientific name
 Cyanoliseus patagonus

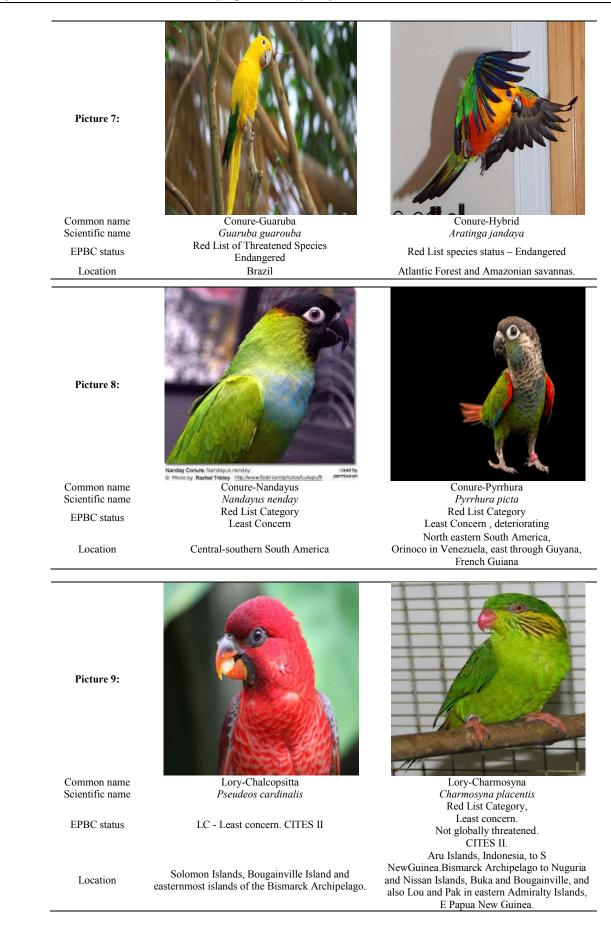
 EPBC status
 Red List species status – Least Concern

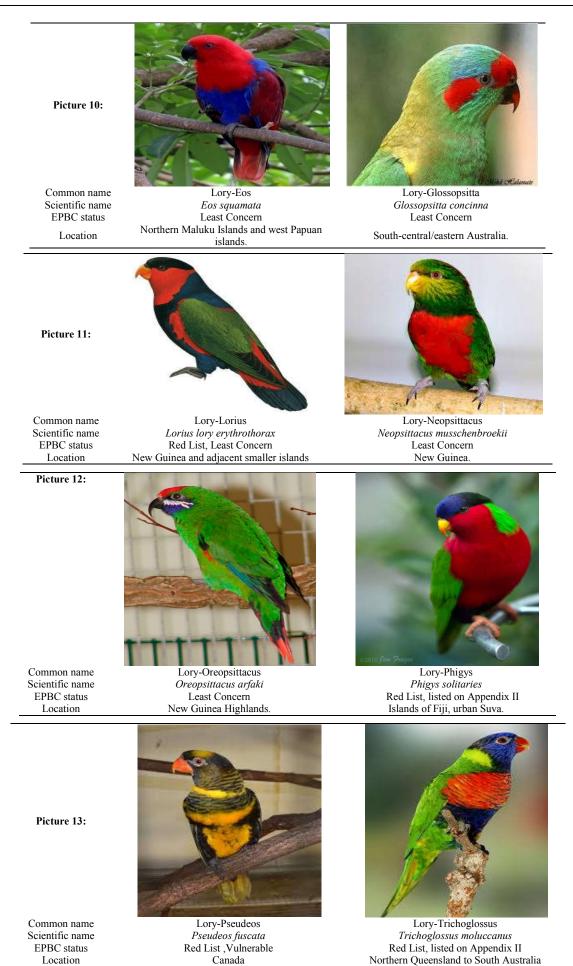
 Location
 Chaco of northern Argentina south to southeast Argentina



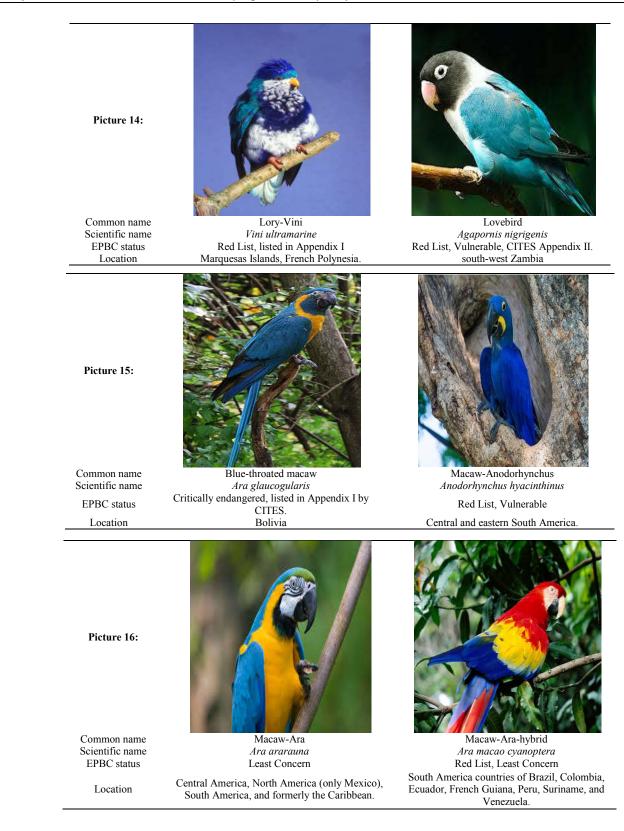
Conure-Enicognathus Enicognathus leptorhynchus Red List Category Least Concern

Lican Ray, Araucanía, Chile.





15353







Picture 19:

Common name

Scientific name

EPBC status

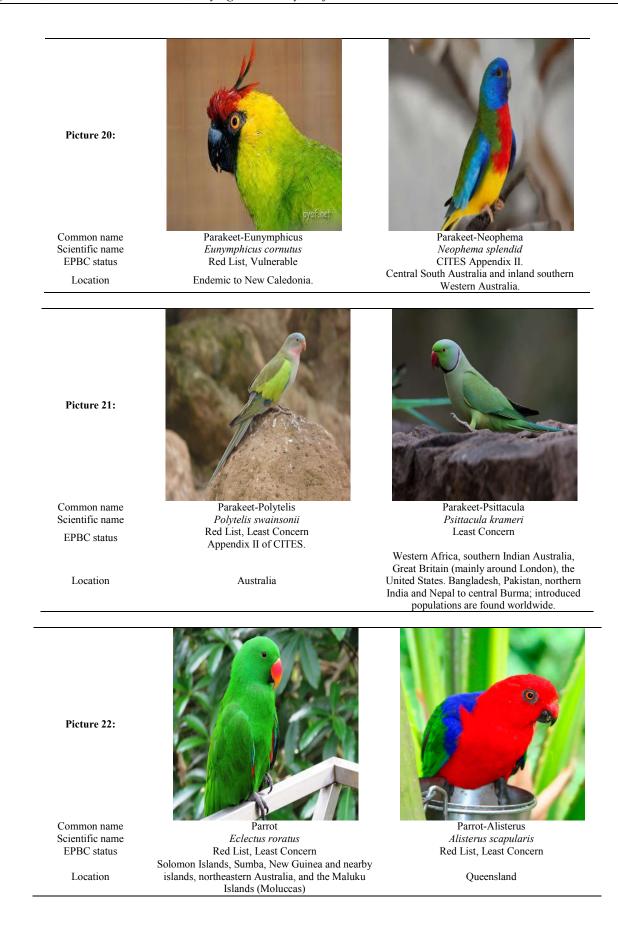
Location

Parakeet-Barnardius Barnardius Zonarius Not globally threatened. CITES II.

South western forests of coastal and subcoastal Western Australia.



Parakeet-Brotogeris Brotogeris versicolurus Red List , Least Concern South America, occupying French Guiana, the Amazon basin from the north of Brazil to the south-east of Colombia, east of Ecuador, north of Argentina and Paraguay and the south-east of Brazil





Common name Scientific name EPBC status Location

Picture 23:

Aprosmictus jonquillaceus threat status: Near Threatened savanna in East Timor and the Indonesian islands of Wetar, West Timor and Roti



Parrot-Coracopsis Coracopsis nigra Red List, Least Concern Comoros, Madagascar, Mayotte, and Seychelles



Picture 24:

Common name Scientific name EPBC status

Location

Parrot-Deroptyus Deroptyus accipitrinus Red List , Least Concern Ecuador, Suriname, Bolivia, Brazil, Colombia, areas of northeast Peru, Venezuela, French Guiana and Guyana.



Parrot-Eclectus Eclectus roratus threat status: Least Concern Solomon Islands, Sumba, New Guinea and nearby islands, northeastern Australia, and the Maluku Islands (Moluccas)

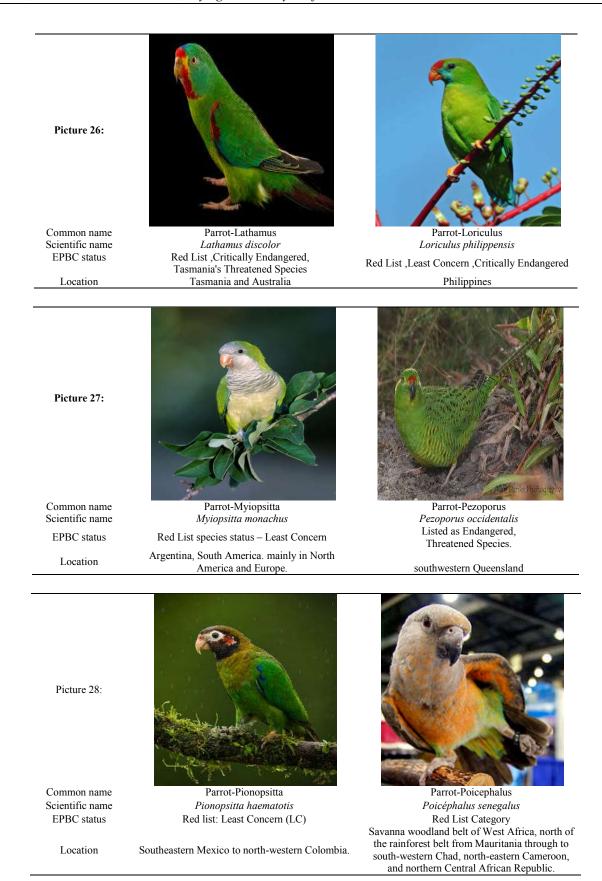
Picture 25:



Common name Scientific name EPBC status Location Parrot-Fig Psittaculirostris edwardsii Red List, Least Concern different regions of New Guinea



Parrot-Prosopeia Prosopeia tabuensis Red List category Fiji, Tonga, islands of Kadavu and Ono.



Picture 29: Parrot-Psittacus Common name Parrot-Psephotus Scientific name Psephotus varius Psittacus erithacus EPBC status Red List Endangered in Queensland Angola; Burundi; Cameroon; Central African Republic; Congo; Congo, The Democratic Location Republic of the; Côte d'Ivoire; Equatorial southern and central Cape York Peninsula Guinea; Gabon; Ghana; Kenya; Nigeria; Rwanda; Sao Tomé and Principe; Tanzania, United Republic of; Uganda Picture 30: Parrot-Psittrichas Common name Parrot-Psittinus Scientific name Psittinus cyanurus Psittrichas fulgidus Red List Category Classified as Near Threatened (NT) on the IUCN EPBC status Red List and listed on Appendix II Vulnerable southern Tenasserim, Myanmar, peninsular Barcelona, Spain and Cambridge, UK. Location Thailand. Picture 31:

Common name Scientific name EPBC status

Location

Parrotlet Forpus coelestis Red List Category LC

Arid littoral of w Ecuador and nw Peru.

Pionus Pionus Pionus Menstruus Red List Category

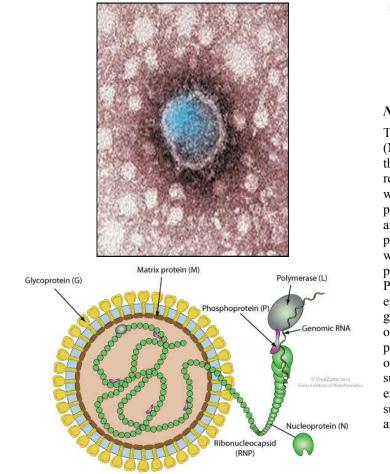
LC subtropical South America and southern Central America, from Costa Rica, Venezuela and Trinidad south to Bolivia and Brazil.



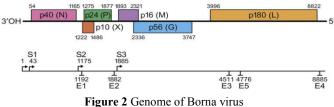
Common name Scientific name EPBC status Location

Picture 32:

Rosella *Platycercus eximius* Red List Category Australia, New Zealand

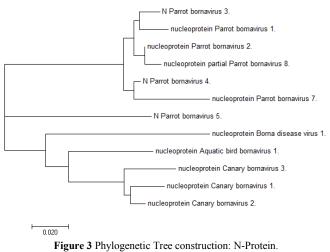


Avian borna viruses are negative-sense, single-stranded, non segmented enveloped RNA viruses, ABV morphology and genome organization are closely similar to the mammalian borna virus.ABV viral particles were 80 to 100 nanometer (nm) in diameter covered with lipid envelope.ABV has six open reading frames (ORF) with a typical 3' to 5'genome organization . The first predicted transcription unit encodes for the viral nucleoprotein (N), the second predicted transcription unit for the regulatory protein (X) and the viral phosphoprotein (P), and the third transcription unit is predicted to code for the viral matrix protein (M), the viral glycoprotein (G) and the RNA-dependent-RNA- polymerase (L). ABV X and P are encoded from overlapping reading frames and the primary transcript of the third transcription unit is likely formed by splicing. Three transcription units (I, II, III) have been determined in the genomic organization of BoDV-1 with three transcription initiation signals (S1–S3) and four transcription termination signals (T1-T4) which 15360arbor at least six open reading frames (ORFs). A 21-22 nucleotide (nt) deletion in the region between the N and X gene have been identified in parrot bornavirus 1 (PaBV-1), parrot bornavirus 2 (PaBV-2), parrot bornavirus 3 (PaBV-3), parrot bornavirus 4 (PaBV-4), parrot bornavirus 5 (PaBV-5), parrot bornavirus 7 (PaBV-7), ABVCanada geese (ABV-CG), and reptile bornavirus. These fragments might have regulatory function for the control of expression of the viral proteins X and P. The loss of these elements in ABV suggests that regulation of the X protein expression may be carried out by other means in ABV-infected cells.



#### Nucleoprotein (N)

The first transcription unit of MRNA encodes a nucleoprotein (N). BoDV-N interacts with the viral RNA and accumulates in the nucleus during the life cycle of the virus. During the viral replication cycle, BoDV-1-N is synthesized like other proteins within the cytoplasm and then enters the nucleus, where it participates in the transcription and replication process. There are two different isoforms of BoDV-1-N (p38 (38 kDa) and p40 (40 kDa). The p40 is primarily located within the nucleus while p38 is mainly expressed in the cytoplasm. ABV-N was present intranuclearly in glial cells of the cerebrum, and Purkinje neurons of the cerebellum, astroglial cells, glial cells, ependymal cells, ganglia of the myenteric plexus in the gastrointestinal tract, cardiomyocytes, tubular epithelial cells of the kidney, epithelial cells of the GIT organs, photoreceptors, smooth muscle cells of the lamina muscularis of the GIT organs, arteries in the heart. Recent studies have suggested that nuclear localization signal (NLS) and/or nuclear export signal (NES) are expressed by the major viral antigens such as N and P are imported to travel between the cytoplasm and nucleus through the nuclear pore complex.



# Evolutionary relationships of taxa (Phylogenetic Tree construction: N-Protein)

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.50946952 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 12 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 113 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

#### Phosphoprotein (P)

The second transcription unit of MRNA encodes a phosphoprotein protein. The 5'-end of the P protein ORF overlaps with the 3'-end of the regulatory X protein. BoDV-P protein is act as cofactor for the RNA-dependent RNA polymerase. ABV-P was present in the nucleus and or cytoplasm in the cells of various organs such as glial cells and neurons of the brain and spinal cord, enteric nervous tissues, cells in the lamina propria of the GIT, cells of the retina, tubular epithelial cells of the kidneys, seminiferous tubules, islet cells of the pancreas and smooth muscle cells of blood vessels in the heart.

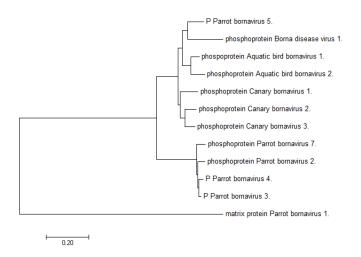


Figure 4 Evolutionary relationships of taxa (Phylogenetic Tree construction: P-Protein).

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 2.64199838 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 12 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 135 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

#### X Protein (X)

The second transcription unit of MRNA encodes a 10 kD a non-structural X protein. BoDV-X is localized mainly in the cytoplasm and nucleus. ABV-X is not a part of the viral RNP in all ABV genotypes. X protein are binds to regulatory components of the molecules to form a viral polymerase complex and interfered with the type I IFN system and type I IFN system.

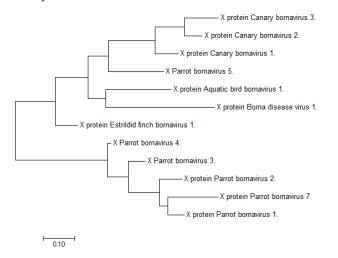


Figure 5 Evolutionary relationships of taxa (Phylogenetic Tree construction: X-Protein)

The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model. The tree with the highest log likelihood (-1131.40) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 12 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 85 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

#### Matrix protein (M)

The third transcription unit of MRNA encodes a 16-kDa (M) non glycosylated BoDV-M protein. The function of BoDV-M protein is binding to RNPs and other viral proteins without blocking the viral polymerase to release mature viral particles during the process of assembly and budding.

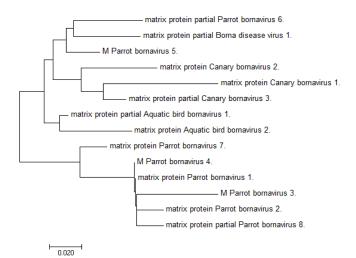


Figure 6 Evolutionary relationships of taxa (Phylogenetic Tree construction: M-Protein).

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.52970782 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 14 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 62 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

#### Glycoprotein (G)

The third transcription unit of MRNA encodes a 56-kD glycol protein (G). Post translational modification mechanisms play an important role in the N-glycosylation configuration changes to increasing their mannose oligosaccharides mass (G precursor). Presence of glycosylation sites which allows the virus to attach to many epitopes on the host cells at C-terminal transmembrane domain. The function of GP–N terminal domain is necessary for virus receptor recognition and cell entry. The GP–C terminal domain transports the materials from the viral capping region to plasma membrane of the host cell.

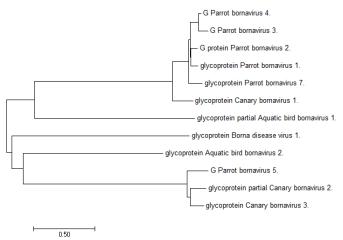


Figure 7 Evolutionary relationships of taxa ( Phylogenetic Tree construction: G-Protein)

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 7.81457092 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 12 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 213 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

#### RNA-dependent RNA polymerase (L)

The nucleus of the infected cells are contains MRNA transcript, the third transcription unit of MRNA encoding a BoDV-L protein (190 kD) and the transcripts that lacks intron I and intron II, serve as messages for expression of the polymerase protein of BoDV.

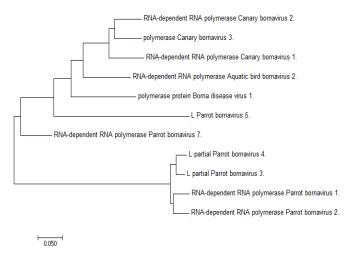


Figure 8 Evolutionary relationships of taxa ( Phylogenetic Tree construction: L-Protein)

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 1.40115646 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 11 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 834 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

#### Psittacine beak and feather disease

Psittacine beak and feather disease is caused by psittacine circo virus (PCV). The virus kills feather and beak cells of psittacine bird. Symptoms of psittacine circoviral disease include diarrhea, feather abnormalities, inflammatory lesions found in the liver, kidney, heart, Pancreas, and skin, high juvenile mortality or long-term immunological suppression. The virus multiplies in the *proventriculus*, liver and spreading out through direct contact with affected birds, ingestion or inhalation of feather dust, dander and faeces. The virus can also be transmitted via contact with contaminated surfaces such as feeding equipment, nesting materials and clothing. Younger birds, particularly neonates, appear to be more susceptible to infection. The circovirus will attack growing feathers (feather loss, abnormal feather colouring, deformed feathers, weak feather attachment, feather calamus abnormalities). The virus is very stable in the environment; it is also resistant to disinfectants. Psittacine birds that develops beak abnormalities (a longer beak, Scissors Beak and Prognathism), necrosis of the hard palate observed in severe cases. Circovirus attack the primary organ (thymus and bursa of *fabricius*) and preventing lymphocyte production.



**Over Growth** 



**Scissors Beak** 



Prognathism



Normal Figure 9 Psittacine beak disease



Abnormal coloration



Normal

Hyperkeratosis and hyperpigmentation



Hyperkeratosis in Cockatoo



Normal

Figure 10 Psittacine Feather disease

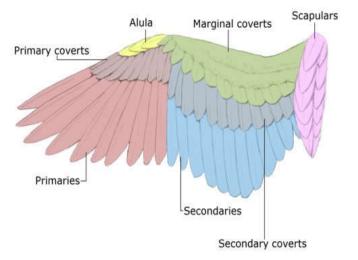


Figure 11 Avian Feather

 Table 1 Scoring system to determine feather condition in parrots (Chest, back, and legs) (Kalmar *et al.*, 2007)

Score	Feathers	Down	Skin	
0	all or most removed	exposed but intact	exposed and injured	
0.25	all or most removed	all or most removed	exposed but not injured	
0.5	all or most removed	some removed	patches exposed	
0.75	all or most removed	exposed but intact	not exposed	
0.75 1 1.25	more than half removed less than half removed more than half removed	some removed some removed exposed but intact	patches exposed patches exposed not exposed	
1.5	less than half removed	exposed but intact	not exposed	
1.75	marked fraying or breakage	not exposed	not exposed	
2	little or no fraying or` breakage	not exposed	not exposed	

Table 2 Scoring system to determine feather condition in	
parrots (Wings) (Kalmar et al., 2007)	

Score	Coverts, primaries (I), and secondaries (II)	Down	Skin
0	all or most coverts, I, and II removed	Removed	exposed and injured
0.5	all or most coverts, I, and II removed	Removed	exposed but not injured
1	more than half of coverts removed	exposed and intact	not exposed
1	more than half of I and II removed	exposed and intact	not exposed
1.5	less than half of coverts removed	exposed and intact	exposed and intact
1.5	less than half of I and II removed	exposed and intact	exposed and intact
1.5	I and II markedly frayed or broken	exposed and intact	exposed and intact
2	little or no fraying or breakage	exposed and intact	exposed and intact

 Table 3 Psittacine Beak and Feather Disease (American association of Zoo Veterinarians infectious disease committee manual- Ritcjie, 2013).

Animal Group(s) Affected	Transmission	Clinical Signs	Severity	Treatment	Prevention and Control	Zoonotic
Psittacines - Old World more than New World species	Direct contact with infected animals with virus presented by inhalation or ingestion.	Peracute: Particularly common in African grey parrots with pancytopenia and death.	World psittacines. Chronic and less severe disease in lovebirds, lories and lorikeets, particularly those birds infected with PCV-2.	Supportive care should be provided in isolated environments where even caretakers have no contact with other birds.	Prevention PCR-based testing has reduced spread in managed populations.	None known
	contact with contaminated	Acute: Depression followed by appearance of dystrophic feathers and death.			Developed vaccine has reached governmental approval stage.	
	systems, for years.	Chronic: Progressive appearance of dystrophic feathers. Necrotic beak nd ulcerations in some long term infected birds. Death occurs in months to years.			Control Testing and isolation of infected birds; strict entry quarantine protocols.	

#### **CONCLUSION**

These studies give a proper knowledge about the identification and implementation of action against to the *Psittacine* Beak and Feather Disease. Research is still required as there are still significant gaps in our knowledge about the virus characteristics, transmission factors, host factors, environment factors, and reservoirs of the virus. PBFD is not considered a threat for most species or more immediate threat. Research on the potential threat of PBFD at species level is required as well as a focus on action across all the species. Communication and information exchange could be better coordinated to be considered in the future management of PBFD.

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