Original Research Article

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Prevalence of *Cryptococcus* species *Naganishia albida* and *Papiliotrema laurentii* in Birds and its Associated Sources from Lower Brahmaputra Valley of Assam

Nurul Islam (1)*, Rupjyoti Bharali (2), Sailen Talukdar (2), Syed Akram Hussain (3), Afzal Hoque Akand (4), Hridip Kumar Sarma (2), Subhash Sarma (5), Chanchal Debnath(6)

[1]District A H and Veterinary Office, AH and Veterinary Department, Govt. of Assam, Kamrup Chenikuthi, Guwahati – 3, Assam,
[2]Department of Biotechnology, Gauhati University, Guwahati-781014, Assam, India, [3]Division of Veterinary Public Health, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Kashmir, Shuhama, Srinagar-19006, India, [4]Division of Veterinary and Animal Husbandry Extension, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Kashmir, Shuhama, Srinagar-19006, India,
[5]Special Animal Husbandry Programme, A H and Veterinary Department, Govt. of Assam, Nagaon, PIN. 782003
[6]Department of Veterinary Public Health & Epidemiology, WBUAFS

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Abstract

Cryptococcus is an encapsulated, yeast-like fungus that can cause respiratory, neurological, and systemic diseases in both humans and animals. This study investigated the distribution of *Cryptococcus* species in pigeon droppings, avian excreta, and soil across six regions of the lower Brahmaputra Valley, Assam, India.Fungal isolates were obtained using Sabouraud Dextrose Agar (SDA) and Bird Seed Agar (BSA), identified by capsule staining and biochemical tests, and confirmed via ITS-1 and ITS-4 sequencing of 18S rDNA.Out of 384 samples, 62 (16.15%) yielded *Cryptococcus* species: 37 (9.64%) *Naganishia albida* and 25 (6.51%) *Papiliotrema laurentii*. These were predominantly found in dry (19.35%) over moist (7.61%) samples, indicating better ecological adaptation to dry environments. *Cryptococcus albidus* was most prevalent in dovecotes (14.11%) and attics (12.36%), while *P. laurentii* showed similar patterns. Notably, *C. neoformans* was absent, suggesting the study area may not support its environmental survival.These findings highlight the ecological and epidemiological importance of non-neoformans species and underscore the need for continued surveillance under the "One Health" framework.

Keyword: *Cryptococcus*, Pigeon droppings, Natural substrates, Ecological relationship, Lower Brahmaputra valley, Prevalence

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Introduction:

Cryptococcus, under *Basidiomycota* family is yeast like fungus causes respiratory, neurological and other systemic diseases in both humans and animals (Littman, 1959). Of more than 70 species, majority thrives in the environment and few of them are medically important disease causing agents (Kwon-Chung et al., 2014). Amongst all species, *Cryptococcus neoformans* is regarded as the major human and animal pathogen, while *Cryptococcus albidus* and *Cryptococcus laurentii* areknown to cause moderate to severe diseases in immuno-compromised individuals (Cheng et al., 2001). Excreta of pigeon act as reservoir of *Cryptococcus* species and reported from various countries (Ruiz et al., 1989). Pigeon droppings are commonly available in most unlikely places like roofs and ventilations of abandoned buildings, cornices, leaves and branches of trees, which serve as an ecological niche for adaptation, dispersion, replication and transmission of *Cryptococcus*. Fruits and vegetables retain the fungi as saprophytes, causing infection in man and animals either by inhalation of spores or through subcutaneous inoculation (Emmons, 1951, 1955; Dave and Pal, 2015).

Cryptococcosis is a highly infectious and enigmatic mycotic disease that affects a variety of animals worldwide (Pal and Randhawa, 1976; Pal, 1997). It causes respiratory as well as neurological problems and is often sporadic in nature (Casadevall et al., 1992). Prevalence of cryptococcosis among AIDS patients stands at 2.33% and 6.8% worldwide and within India, respectively. Incidentally, the exact epidemiological data on the incidence and prevalence of the disease are not easily available as the disease is not a notifiable one, even though morbidity and mortality in humans as well as in animals are well documented (Mitchell and Perfect, 1995).

In India, Cryptococcosis was documented by Rao and Lailauwala (1952) and subsequently many other investigators reported its occurrence in various parts of the country (Sinha and Barua, 1960; Aikal et al., 1967; Talwar and Meera, 1986); but still literatures are scanty on the non-neoformans species like *Cryptococcus albidus, C. laurentii*or *C. uniguttulatus* (Fell and Statzell-Tallmann, 1989; Ikeda et al., 2002). In recent years, a burst of increase in opportunistic infection by these non-neoformans pathogenic yeasts have been observed (Mc

Curdy and Marrow, 2003; Rosario et al., 2005). Environmental sources including canopy leftovers from some trees are regarded as the main sources of *C. albidus, C. laurentii,* and *C. uniguttulatus* that lead to infection in human and animals (Bernardo et al*.,* 2001; Jang et al*.,* 2011; Kamari et al*.,* 2017).

The epidemiology of non-Neoformans *C. albidus, C. laurentii, C. uniguttulatus, C. luteolus*, and other species is highly relevant, as these species often turn out to be pathogenic and thereby increase the risk of infection (Liu et al., 2014). Several pathological conditions like keratitis (Huang et al., 2015); pulmonary Cryptococcosis due to in diabetic patient and patients suffering from ganglio-neuroblastoma were reported to be caused by *C. laurentii* (Averbuch et al., 2002) and Shankar et al*.* (2006). Reports are also available on Cryptococcal meningitis, Cryptococcal myelitis, and multiple skin lesions in HIV patientsinfected with *C. neoformans* var. *grubii* from Assam having a history of occupational exposure to pigeons and chicken (Rai et al*.,* 2014). Such findings are of greater significance and require proper surveillance, along with characterization, to identify the hot spots of *Cryptococcus* occurrence in the lower Brahmaputra Valley of Assam.

Materials and Methods:

In the present investigation, a total of 384 samples of pigeon droppings and droppings of other avian species, as well as environmental soil from different sources from five different distinct regions of the lower Brahmaputra Valley of Assam. Samples collected were transported to the laboratory in ice-cooled boxes, packed and processed on the same day or stored at 4^0Cfor isolating *Cryptococcus* species as per standard methods mentioned elsewhere with slight modifications (Criseo, 1995; Manoharan et al*.,* 2001) in the department of Biotechnology, Gauhati University. Basic environmental characteristics of the collection areas were noted to study the ecological and epidemiological relevance of the organism (Table 1, Figure1).

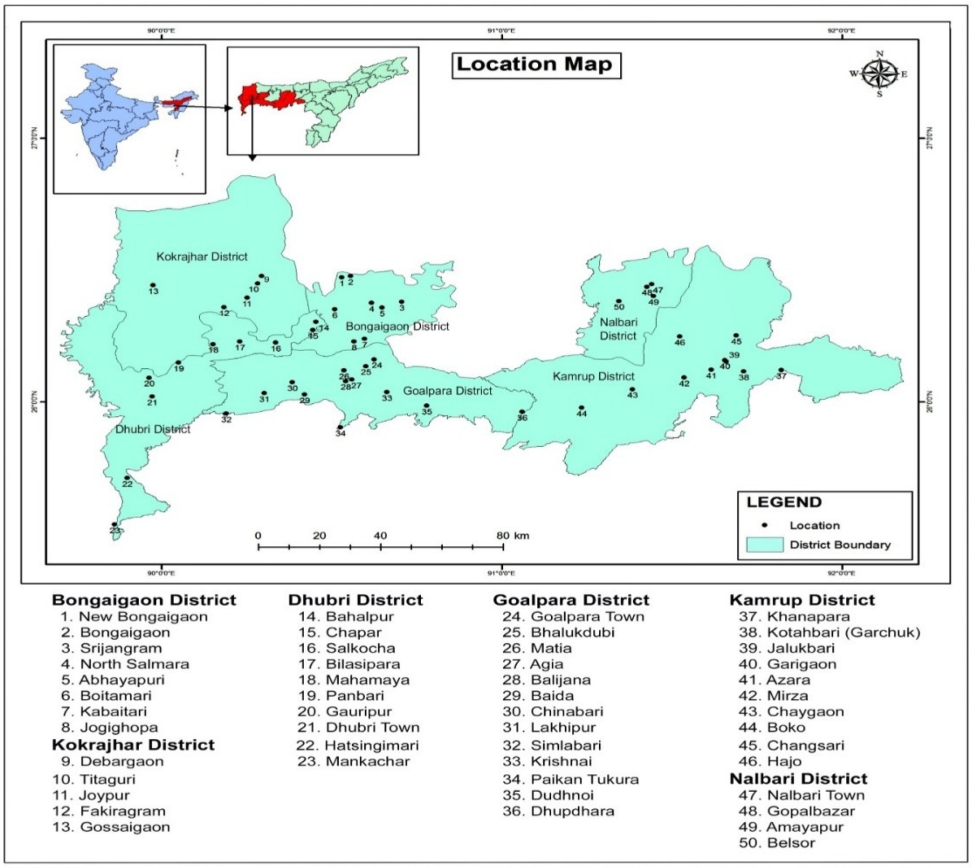


Figure 1: Representation of the sample collection area

Isolation and Identification

For isolation, 2g of each sample was mixed with 20ml sterile physiological saline, vortexed for 5 minutes, and left to settle for 15–20 minutes. One ml of the supernatant was transferred to 9 ml saline with chloramphenicol (0.05 mg/ml) and incubated at 37°C for 1 hour. Then, 0.1 ml aliquots were streaked onto duplicate plates of Sabouraud

Dextrose Agar (SDA) and Bird Seed Agar (BSA), both with chloramphenicol, and incubated at 37°C and 25°C for up to 15 days, with daily observation from day 2.Brown-pigmented colonies on BSA indicated *C. neoformans*, while cream-colored, smooth, mucoid colonies were identified as non-*neoformans* species. *C. laurentii* MTCC 2898 was used as a control. Further identification was confirmed through morphological, cultural, and biochemical tests as per standard protocols.

Capsule Identification

Smear prepared from inoculum stained with India ink and observed under a bright field microscope and phase contrast microscopy as per Kwon-Chung and Bennett (1992). Presence of a distinct, wide, round to oval gelatinous capsule, with or without hyphae, was considered a positive observation.

Molecular Characterization

DNA extraction

DNA extractions from the isolates from pure cultures were done as per the standard protocol described by Ferrer et al., 2001.

PCR amplification

For characterization of *Cryptococcus* species, isolated DNA was amplified in a gradient PCR (Eppendorf Nexus Gradient) following the conditions mentioned by White et al. (1990) with minor modifications. The primers used for amplification included the D1/D2 regions targeting ITS1 and ITS4 with an expected fragment length of 600bp. Details of the primers are given in Table 2. The amplicons were later stored at -20°C for further analysis.

Table 2. Primers used for PCR amplification

Primer	Sequence
ITS1	3'- TCC GTA GGT GAA CCT GCG G -5'
ITS4	5'- TCC TCC GTC TAT TGA TAT GC -3'

Sequencing

Sequencing of PCR products was done at Xcelris Labs Limited, India. Chromatogram files obtained were analyzed for nucleotide-BLAST on the NCBI portal for identification of the species. Phylogram and dendrogram were prepared using PhyML (Phylogenetic Maximum Likelihood) (http://www.atgc-montpellier.fr/phyml/).

Statistical analysis

A brief descriptive analysis was carried out to interpret the data. However, positive results obtained from different sources were compared using the k-proportion test using Monte Carlo / Marascuilo methods to assess their homogeneity across sources with the help of XLSTAT software.

Results and Discussion:

In the present study, a total of 62 *Cryptococcus* species were recovered with a prevalence of 16.15%. Out of 62 positive isolates, 37(9.64%) were identified as *C. albidus* (*N. albida*) (Table 3, Figure 2D, E, F) while 25 (6.51%) were identified as *Cryptococcus laurentii* (*P. laurentii*) (Table 3, Figure 2B, C). Cultural and biochemical characteristics of *N. albida* and *P. laurentii* were presented in (Figure 2B, C) and comparable with the reference strain MTCC 2898 (Figure 2A). Based on the type of sample (dry and moist), *N. albida* (*C. albidus*) and *P. laurentii* (*C. laurentii*) could be isolated from 54 (19.35%) of dry and 8 (7.61%) of moist specimens collected from different geographical niches/co-ordinates (Table 3, Figure 5).

In the study, 57 (22.62%) *Cryptococcus* species isolated from pigeon droppings from sites A and B, 34 (13.49%) were identified to be *C. albidus,* while 23 (9.13%) represented *C. laurentii*. The highest recovery percentage of *Cryptococcus* (57) was observed in pigeon droppings, and the least (2) was from avian species other than pigeons. In terms of relative isolation of positive cultures of *Cryptococcus*, 14 (15.73%) were from attics/ventilations / old and abandoned buildings (Site A), 43 (26.38%) were from houses of pigeon fanciers/dovecots (Site B), 3(4.84%) were from contaminated environment/soil (Site C), while 2 (2.86%) were from other avian sources like chicken, duck, parrot etc. (Site D). The results are presented in Table 3.

Relative and absolute percentage of isolates of *Cryptococcus* (*N. albida* and *P. laurentii*) from each collection site was also analyzed. Prevalence rate of isolates from Site A (3.65%), Site B (11.20%), Site C (0.78%), and Site D (0.46%) was recorded (Table 4). Results interpret that there exists a significant difference at 0.05% among the variants of different proportions obtained from various samples during the study. Values having the superscript across different rows do not differ significantly.

The overall prevalence of *N. albida* across all dry and most environmental samples was 9.64% (37 of 384). Details of the prevalence of *N. albida*is are presented in Table 5, whilst morphological features of the species are presented in Figure 2 (D, E, F) as well as Figure 4B, and Figure 6, respectively. A significant difference at 0.05 % level among the different proportions obtained from different sources during the study. On the contrary, the overall prevalence of *P. laurentii* in all dried and moist environmental samples was 6.51% (25 of 384), 7.89% (22 of 279), and 2.86% (3 of 105), respectively, from different sites. Details of the prevalence of the speciesis presented in Table 6, Figure 4A, and Figure 7, alongwith the comparable datasheet of the reference strain (*C.*

laurentii MTCC 2898, Figure 3), respectively. The results correlate a significant difference at 0.05% level among the representative proportions obtained from different sources during the study. Details of the genetic identity of the isolated strains of *N. albida* and *P. laurentii* of the sequences are presented in Table 7.

Comparisons about the prevalence of *N. albida* and *P. laurentii* across the studied geographical locations depict an overall 11 (20.75%), 15 (18.07%), 8 (16.66%), 15 (16.13%). 8 (13.34%) and 5 (10.64%) numbers of positive isolates representing the districts of Bongaigaon, Dhubri, Kokrajhar, Goalpara, Kamrup and Nalbari respectively (Table 8; Figure 8). The prevalence of pathogenic *C. neoformans* could not be observed from any samples may be due to unfavorable habitat niche for the species in study area.

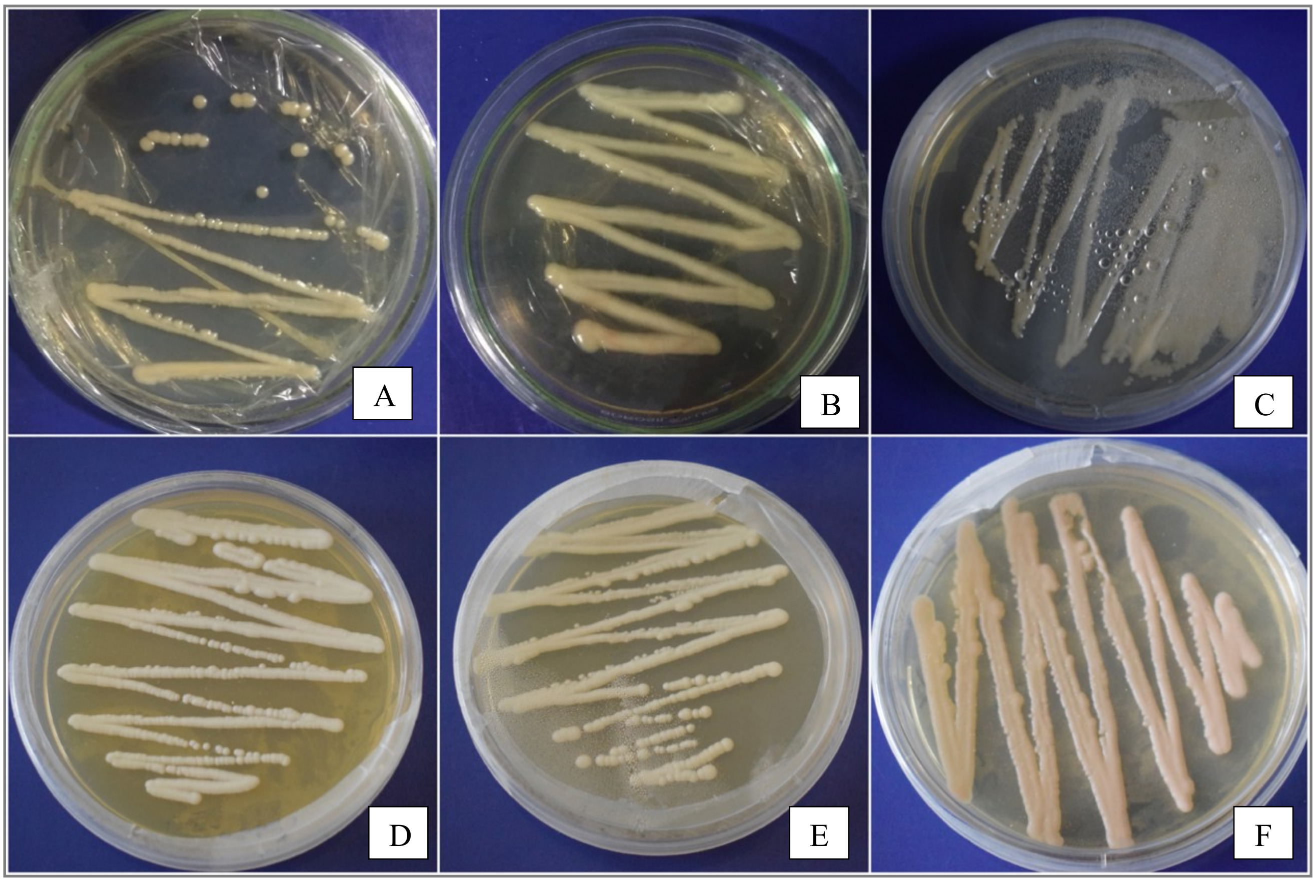


Figure 2: (A) *Papiliotrema laurentii* (MTCC 2898); (B, C) *Papiliotrema laurentii*; (D, E, F) *Naganishia albida*

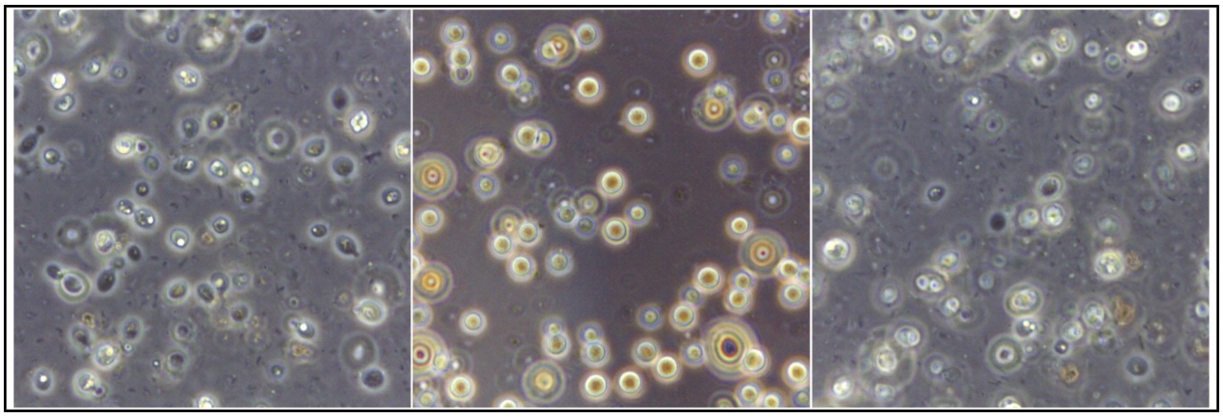


Figure 3: Phase contrast microscopy of reference sample *Papiliotrema laurentii* (MTCC 2898), under 100X magnification

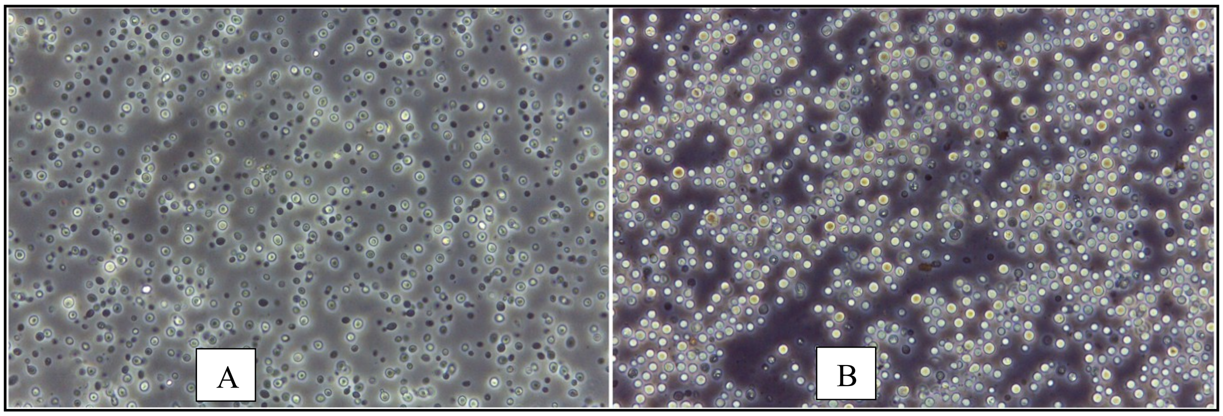


Figure 4: Phase contrast microscopy of (A) *Papiliotrema laurentii*(B) *Naganishia albida* under 40x magnification.

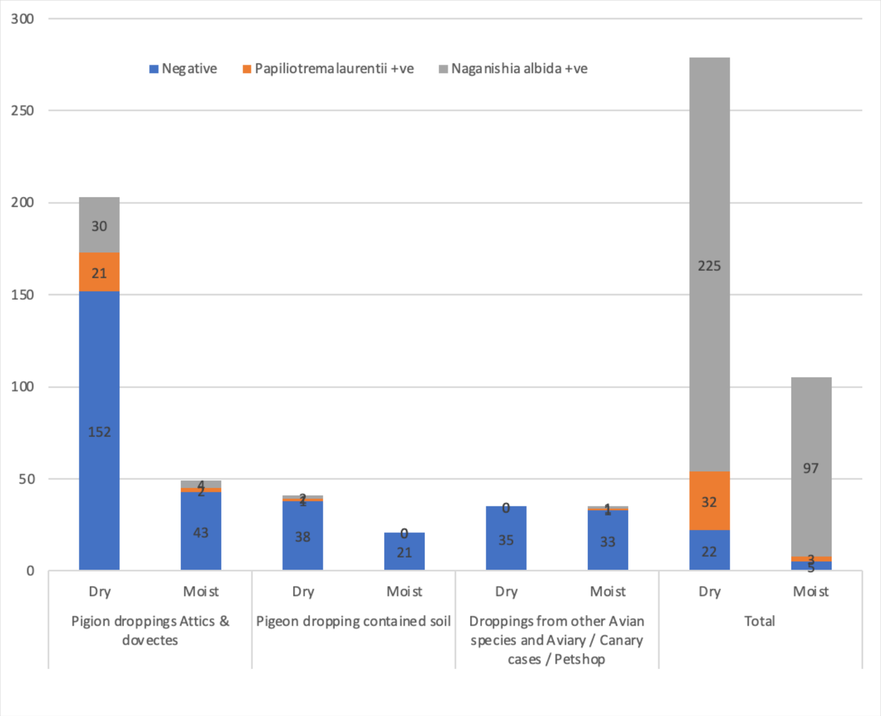
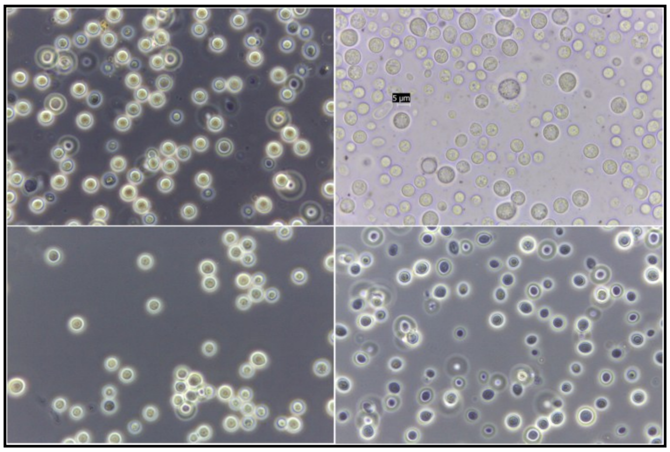


Figure 5: Distribution of positive samples of *Cryptococcus* species



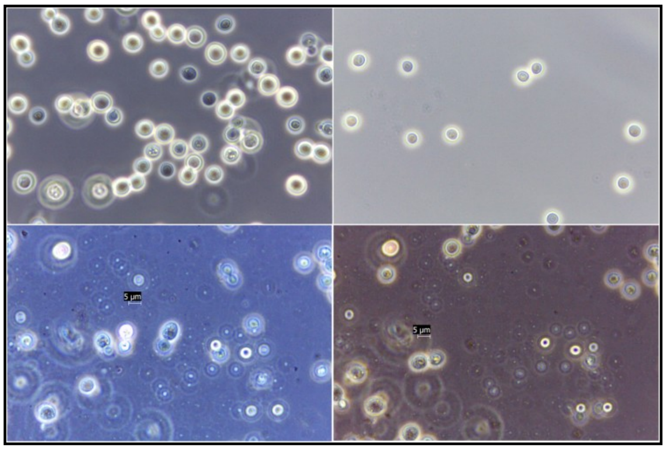


Figure 6: Phase contrast microscopy of *Naganishia albida* under 100x magnification

Figure 7: Phase contrast microscopy of *Papiliotrema laurentii* under 100x magnification.

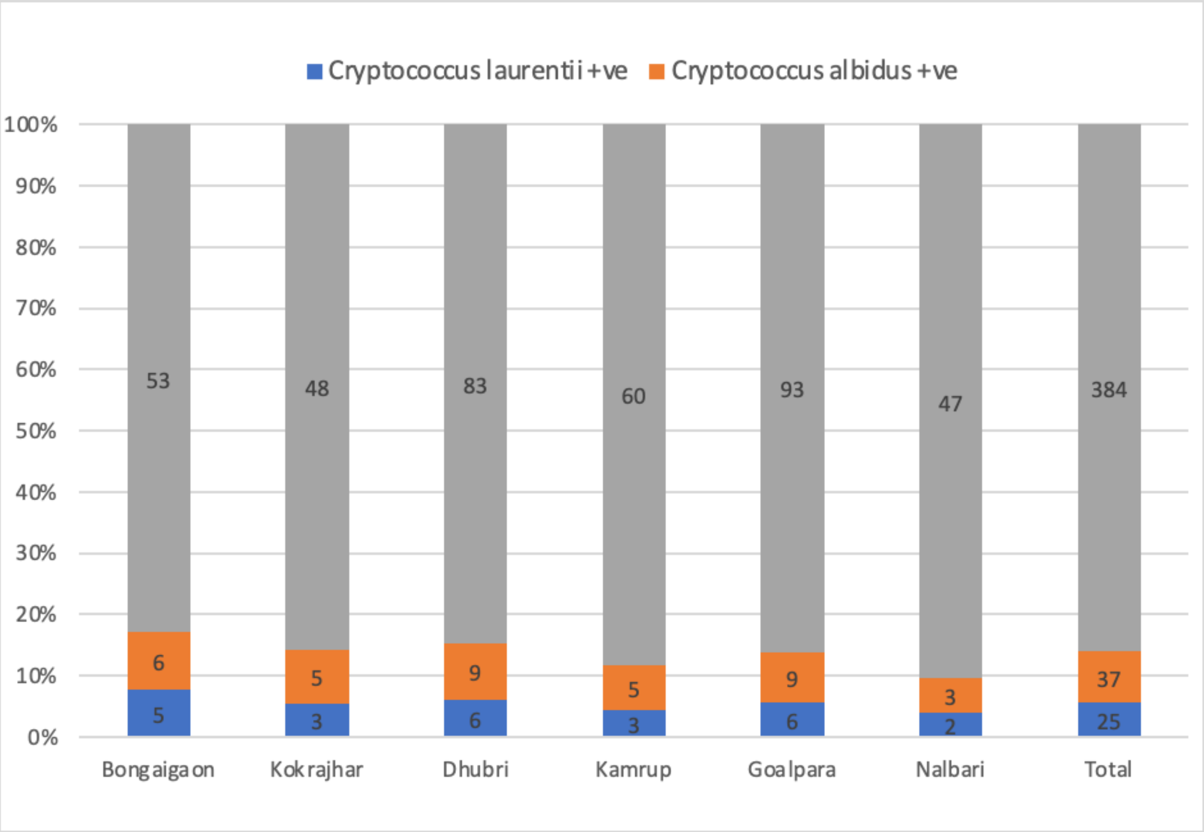


Figure 8: District-wise distribution of positive isolates of *Cryptococcus* species

Table 8: Geographical co-ordinates and environmental niches-wise frequency distribution of positive isolates of *Cryptococcus* species

Sl No.	**Geographical region**	**Total Sample**	***Naganishia albida* positive (nos.)**	***Papiliotrema laurentii* positive (nos.)**	**Total positive sample (nos.)**	**Relative % in the region**	**Absolute % in the region**
1	BONGAIGAON	53 (BNG 1-53)	6	5	11	20.75	2.86
2	KOKRAJHAR	48 (KJR 1-48)	5	3	8	16.66	2.08
3	DHUBRI	83(DHB 1-83)	9	6	15	18.07	3.90
4	KAMRUP	60 (KRP 1-60)	5	3	8	13.34	2.08
5	GOALPARA	93 (GLP 1-93)	9	6	15	16.13	3.90
6	NALBARI	47 (NLB 1-47)	3	2	5	10.64	1.30
					P = 0.762 (>0.05) : NS		
		TOTAL = 384	Total = 37	Total = 25	62	16.15 %	

Cryptococcosis is a major public health concern worldwide, including India. Epidemiology and pathogenicity of this enigmatic myco-zoonosis in man and animals have been documented (Pal, 1986; Mitchel and Perfect, 1995; Pal, 1996). Ecological relationship of *Cryptococcus* was not known until Emmons (1955) found the fungus in droppings of pigeons and soil, followed by subsequent research (Littman, 1959; Pal and Baxter 1985; Pal, 1997; Rosario et al., 2005; Dave and Pal, 2015; Pal, 2015).

Although many studies have been conducted on the epidemiology and pathogenicity of *C. neoformans*, studies on non-neoformans species are scanty. The present study defines the incidence of non-neoformans species of the genus *Cryptococcus* inherent in pigeon droppings and other environments along the lower Brahmaputra valley of Assam.

The study confirmed the presence of two non-neoformans species, viz., *N. albida* and *P. laurentii*. To our knowledge, this is the first report on the isolation of these two species from pigeon droppings and other environmental sources of the northeastern region of India; however, *C. neoformans* could not be recovered. The overall prevalence of *Cryptococcus* spp. was 16.15% (62) retrieved from all six geographical regions, of which 9.64% (37) and 6.51% (25) were identified to represent *N. albida* and *P. laurentii,* respectively. The results are comparable with other findings on isolation of the organism from pigeon droppings, pigeon nests, and eucalyptus tree specimens in China and Iran (Jang et al., 2011; Kamari et al*.,* 2017).

The current findings revealed 21 (10.34%) positive isolates of *P. laurentii* in the droppings of pigeons in dovecotes /fanciers. Similarly, a 20.60% positive isolation rate of *Cryptococcus* in 6 out of 186 pigeon droppings in attics was reported earlier by Kamari et al. (2017). The variation of the findings might be due to differences in climate, humidity, temperature, and other biotic or abiotic factors characteristic of the study area.

In the current study, 13.49% (34) and 9.13% (23) of *N. albida* and *P. laurentii* could be recovered from pigeon droppings from attic ventilations of old buildings, dovecotes, houses of pigeon fanciers, and from cloacal swabs. Several studies also reported the isolation rate of *C. albidus* and *C. laurentii* from droppings of pigeons (Shyamsundar, 2009). At the same time, 4(16%) out of 25 dried plant materials were found to harbor *C. neoformans* in a study conducted at North Chennai (Shyamsundar, 2009).

Although *C. neoformans* is regarded as the most commonly occurring species of the *Cryptococcus*, its absence in the study area reflects the status of overall persistence of the species under humid conditions exposed to abrupt changes in temperatures etc. The effect of seasonal variations on the persistence of non-neoformant *Cryptococcus* isolates cannot be nullified, which requires further investigations through a molecular approach.

While studying the frequency of isolation in the context of regional characteristics (Nalbari and Bongaigaon region), no specific trend in terms of frequency was observed this may be due to the close proximity of the study areas that share the same environmental conditions. Ecological relevance of the non-neoformans species of *Cryptococcus* (4.84% in soil) was the most important finding of this study, with profound influence on replication and transmission of the pathogens to the susceptible population. Such a situation is very imminent and highly risky for crowded populated areas like Nalbari and the Kamrup district of Assam.

Conclusion:

This investigation concludes that *N. albida* and *P. laurentii* are the most prevalent *Cryptococcus* species in the lower Assam region. The absence of *C. neoformans* in the study area highlights the need for extensive surveillance to understand its epidemiological pattern in relation to changing environmental conditions. These findings underscore the ecological and epidemiological significance of *Cryptococcus* infections and emphasize the importance of monitoring and prevention strategies within the framework of the "One Health" approach.

Conflicts of Interest:

No conflict of interest among the authors.

Data availability:

Data will be available on request.

Authors' contribution:

All authors contributed for the study as and when they have required their need.

Ethical approval:

Not applicable.

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***Corresponding author's email ID:** drnurul68@rediffmail.com

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Table 1: Frequency distribution of samples as per their sources and types

BREAKUP OF SAMPLES FROM DIFFERENT SOURCES AND GEOGRAPHICAL REASONS

1	2	3	4	5	6 and 7										
					Source of Samples										
					Pigeons Droppings (A)				**Other Avian Species (Chicken, Ducks etc.) (C)**		**Aviary/ Canary cages/ Pet Shop (D)**		**Soil contaminated with droppings of Pigeons (E)**		
Sl No	**Sample Code**	**Area/ Region**	**Geographical Location/ Place of Sampling**	**Season/Month/ Date**	**Attics, /roofs ventilators of old buildings (A)**		**Pigeon fanciers households (Cloacal/Fresh) (B)**								
					Dry	**Moist**	**Dry**	**Moist**	**Dry**	**Moist**	**Dry**	**Moist**	**Dry**	**Moist**	
1	BNG	Bongaigaon	Jogighopa and NSalmara, Abhyapuri, Baitamari,Bongaigaon Town, Kabaitari, Chalantapara	July, August, September, October/2018	8	2	18	5	3	3	2	2	7	3	Dry-38 Moist-15 = 53
2	KJR	Kokrajhar	Jaipur, KokrajharTown, Titaguri, Devorgaon	July, August, September/2018	8	4	15	6	2	2	2	2	4	3	Dry-31 Moist-17 = 48
3	DHB	Dhubri and South Salmara Mankachar	Bahalpur, Chapar, Bilashipara, Guripur, Dhubri Town, Hatsingimari (Meghyalaya Boarder), Mahamaya	June, July, September, October/2018	21	3	29	8	3	2	3	3	7	4	Dry-63 Moist-20 = 83
4	KRP	Kamrup Rural and Urban	Mirza,Kotabari Gorchuk, Azara,Hajo, Jhalukbari, Chaygaon, Boko	June, July, August, September, October/2018	11	4	19	4	3	1	3	4	7	4	Dry-43 Moist-17 = 60
5	GLP	Goalpara	Lakhipur, Simlabari Tikrikilla Meghyalaya boarder,Agia, Krishnai, Dudhnoi,Matia, Dhupdhara and Goalpara	June, July, August, Septmber/2018	19	3	34	7	4	3	4	5	10	4	Dry-71 Moist-22 = 83
6	NLB	Nalbari	Town area, Gopal Bazar, Belsor, Chamata, Amayapur.	Sep, Oct/2018	5	1	16	2	3	4	3	4	6	3	Dry-33 Moist-14 = 47

Total	72	17	131	32	18	15	17	20	41	21	Total Dry-279 Moist-105 Total = 384

Table 3: Frequency distribution of positive samples of *Cryptococcus* species (pooled sample)

Species of *Cryptococcus*	**Pigeon droppings with code no.**									**Pigeon droppings contaminated with soil (C)**			**Droppings from other Avian species and Aviary/Canary cases/Pet shop (D)**			**Total positive samples from all sources (E)**		
	(A)			**(B)**			**(A+B)**											
	Attics/Ventilators/roofs of private and public / Old/abandoned buildings (nos and %)			**Houses of Pigeons fanciers/dovecotes (nos and %)**			**Pigeons dropping Attics and dovecotes (nos and %)**			**nos and %**			**nos and %**			**nos and %**		
	Dry	**Moist**	**Total**	**Dry**	**Cloacal/ Moist**	**Total**	**Dry**	**Moist**	**Total**	**Dry**	**Moist**	**Total**	**Dry**	**Moist**	**Total**	**Dry**	**Moist**	**Total**
Total nos of samples	72	17	89	131	32	163	203	49	252	41	21	62	35	35	70	279	105	384
N. albida (positive and %)	10 (13.89)	1 (5.88)	11 (12.36)	20 (15.27)	3 (9.37)	23 (14.11)	30 (14.78)	4 (8.16)	34 (13.49)	2 (4.88)	0 (0.00)	2 (3.22)	0 (0.00)	1 (2.85)	1 (1.42)	32 (11.46)	5 (4.76)	37 (9.64)
P. laurentii (positive and %)	3 (4.17)	0 (.00)	3 (3.37)	18 (13.74)	2 (6.25)	20 (12.27)	21 (10.34)	2 (8.08)	23 (9.13)	1 (2.44)	0 (0.00)	1 (1.61)	0 (0.00)	1 (2.85)	1 (1.42)	22 (7.89)	3 (2.86)	25 (6.51)
N. albida and *P. laurentii* (positive and %)	13 (18.05)	1 (5.88)	14 (15.73)	38 (29.07)	5 (15.62)	43 (26.38)	51 (25.12)	6 (12.24)	57 (22.61)	3 (7.32)	0 (0.00)	3 (4.84)	0 (0.00)	2 (5.71)	2 (2.85)	54 (19.35)	8 (7.61)	62 (16.15)

Table 4: Isolates of *Cryptococcus* Species *Naganishia albida* (*C. albidus)* and *Papiliotrema laurentii* (*C. laurentii)* from different sources of samples

			Dry			Moist			Total		
Sl No	**Collection site**	**Environmental niches**	**n**	**Positive**	**Relative and absolute isolations/ isolations (No and %)**	**n**	**Positive**	**Relative and absolute isolations/relative isolations (No and %)**	**n**	**Positive**	**Relative and absolute isolations/relative isolations (No and %)**
1	Attics / ventillations of old / abandoned / building (A)	Pigeons' droppings (*C livia*)	72	13	18.05 (4.11)	17	1	5.88 (0.84)	89	14^{ab}	15.73 (3.22)
2	Houses of Pigeons' fancies/dovecotes (B)	Pigeons' droppings (*C livia*)	131	38	29.00 (12.02)	32	5	15.62 (4.20)	163	43^{b}	26.38 (9.89)
3	Environmental samples/ soil (C)	Contaminated with pigeons' droppings	41	3	7.32 (0.95)	21	0	0	62	3^{a}	4.84 (0.69)
4	Other birds (D)	Parrots, chickens, ducks etc.	35	0	0.00	35	2	5.71 (1.66)	70	2^{a}	2.86 (0.46)
											P = 0.001 (<0.05) *
5	Samples Total (E)		**279**	**54**	**19.35**	**105**	**8**	**7.61**	**384**	**62**	**16.15**

Figures in parenthesis indicate percent with respect to total sample size (N), n = number of samples

Table 5: Isolates of *Naganishia albida* (*C. albidus)* from different sources of samples

			Collected samples/ Positive isolates						Absolute isolations/relative isolations (No and %)		
			Dry			**Moist**			**Total**		
Sl No	**Collection site / Sources**	**Environmental niches**	**n**	**Positive**	**Relative and absolute isolations/ isolations (No and %)**	**n**	**Positive**	**Relative and absolute isolations/ isolations (No and %)**	**n**	**Positive**	**Relative and absolute isolations/ isolations (No and %)**
1	Attics / ventillations of old / abandoned / building (A)	Pigeons' droppings (*C livia*)	72	10	13.89 / 3.58	17	1	5.88/0.95	89	11^{ab}	12.36 / 2.86
2	Houses of Pigeons' fancies/dovecotes (B)	Pigeons' droppings (*C livia*)	131	20	15.27 / 7.16	32	3	9.37/2.86	163	23^{b}	14.11 / 5.99
3	Environmental samples/ soil (C)	Contaminated with pigeons' droppings	41	2	4.88 / 0.717	21	0	0.00	62	2^{a}	3.23 / 0.52
4	Other birds(D)	Parrots, chickens, ducks etc.	35	0	0.00	35	1	2.85/0.952	70	1^{a}	1.43 / 2.60
											P = 0.008 (<0.05) *
5	Samples Total (E)		**279**	**32**	**11.46**	**105**	**54**	**3.80**	**384**	**37**	**9.64**

Figures in parentheses indicate percent with respect to total sample size (N), n – indicates no of samples. (Values having the superscript across different rows do not differ significantly)

Table 6: Isolates of *Papiliotrema laurentii* (*C. laurentii)* from different sources of samples

Sl No	Collection site / Sources	Environmental niches	Collected samples/ Positive isolates						Absolute isolations/relative isolations (No and %)		
			Dry			Moist			Total		
			n	Positive	Relative and absolute isolations/ isolations (No and %)	n	Positive	Relative and absolute isolations/ isolations (No and %)	n	Positive	Relative and absolute isolations/ isolations (No and %)
1	Attics / ventillations of old / abandoned / building (A)	Pigeons' droppings (*C livia*)	72	3	4.17/1.075	17	0	0.00	89	3^{ab}	3.37/0.781
2	Houses of Pigeons' fancies/dovecotes (B)	Pigeons' droppings (*C livia*)	131	18	13.74 / 6.45	32	2	6.25 (1.904)	163	20^{b}	12.27 / 5.20
3	Environmental samples/ soil (C)	Contaminated with pigeons' droppings	41	1	2.44 / 0.359	21	0	0.00	62	1^{a}	1.61 / 0.26
4	Other birds (D)	Parrots, chickens, ducks etc.	35	0	0.00	35	1	2.85 (0.952)	70	1^{a}	1.42 / 0.26
										P = 0.001 (<0.05) *	
5	Samples Total (E)		**279**	**22**	**7.89**	**105**	**3**	**2.86**	**384**	**25**	**6.51**

Figures in parenthesis indicate percent with respect to total sample size (N), n – indicate no of samples. (Values having the superscript across different rows do not differ significant)

Table 7: Sequence data of *Cryptococcus* species isolated from different sources

Sl No	Laboratory Code	Source	Spacies identified (isolates)	Accession No	Sequence (in FASTA format)
1	GLP 19	Pigeon (*Columba livia*) dropping	*Naganishia albida (Cryptococcus albidus)*	MT 102389	>MT102389.1 Naganishia albida isolate GLP19 small subunit ribosomal RNA gene, partial sequence TTCTGGTGCCCAAGCAGCCGCGGTAATTCCAGCTCCAATTAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTA GTTGAACTTCAGGCCCGACGGGGTGGTCTGCCTCACGGTATGTACTATCCGGTTGGGCCTTACCTCCTGGTGAGCCC GTATGTCGTTTACTCGGTGTGCGGGGGAACCAGGAATTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCATATG CCCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTCTAGGATCGCCGTAATGAT TAATAGGGACGGTTGGGGGCATTAGTATTCAGTTGCTAGAGGTGAAATTCTTAGATTTACTGAAGACTAACTACTGC GAAAGCATTTGCCAAGGACGTTTTCATTAATCAAGAACGAAGGTTAGGGGATCAAAAATGATTAGATACCGTTGTAG TCCTAACAGTAAACTATGCCGACTAGGGATCGGGCCATGTTCAACTTTTGACTGGCTCGGCACCTTACGAGAAATCA AAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGT GGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTGATAG CTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTTAATTCCGATA ACGAAACGAGACCTTTAACCTTGCGTTAGAATTAGGACCCCGGGTTCAGGGGACCT
2	DHB 39	Environmental sample near dovecotes (contaminated soil)	*Papiliotrema laurentii* (*C. laurentii*)	MT 102618	>MT102618.1 Papiliotremalaurentii isolate DHB39 small subunit ribosomal RNA gene, partial sequence TTTCTGGTGCCGCAGCAGCCGCGGTAATTCCAGCTCCAGTAGCGTATATTAAAGTTGCTTGCAGTTAAAAAGCTAGT AGTCGAACTTCGGCCCTGGCTGGACGGTCCGCCTTACGGTGTGCACTGTCCGGCCGGGTCTTACCCTCTTGGTGAGG CCGCATGCCCTTTACTGGGTGTGCGGTGGAACCAGGAATTTTACCTTGAGAAAATTAGAGTGTTCAAAGCAGGCATT TGCCCGAATACATTAGCACGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTCTAGGATCGCCGTAATG

					ATTAATAGGGACGGTCGGGGGCATTAGTATTCCGTTGCTAGAGGTGAAATTTTTAGATTTACGGAAGACTAACTTCTGCGAAAGCATTTGCCAAGGACGTTTTCATTGATCAAGAACGAAGGTTAGGGGATCAAAAACGATTAGATACCGTTGTAGTCTTAACAGTAAACTATGCCGACTAGGGATCGGGCCACGTTAATTTCTGACTGGCTCGGCACCTTACGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGTGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTGATAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGATAACGAACGAGACCTTAACCTGCTAAATAGCCAGGCCGGCTTTGGCTGGTTCCGGTACTTCTTTTACTGGACTGTACGTCTATTATACCCACACTGGATCTGGGACTAACCGCCGGTCGCTAGATCCCCCCTATGAGA
3	BNG 4	Pigeon (*Columba livia*) dropping	*Papiliotrema laurentii* (*C. laurentii*)	MT 102623	>MT102623.1 Papiliotremalaurentii isolate BNG4 small subunit ribosomal RNA gene, partial sequence GCGGTAATTCCAGCTCCAGTAGCGTATATTAAAGTTGTTGCAGTTAAAAGCTCGTAGTCGAACTTCGGGCCTGGCTGGACGGTCCGCCTTACGGTGTGCACTGTCCGGCCGGGTCTTACCTCTTGGTGAGGCCGCATGCCCTTTACTGGGTGTGCGGTGGAACCAGGAATTTTACCTTGAGAAAATTAGAGTGTTCAAAGCAGGCATTTGCCCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTCTAGGATCGCCGTAATGATTAATAGGGACGGTCGGGGGCATTAGTATTCCGTTGCTAGAGGTGAAATTCTTAGATTTACGGAAGACTAACTTCTGCGAAAGCATTTGCCAAGGACGTTTTCATTGATCAAGAACGAAGGTTAGGGGATCAAAAACGATTAGATACCGTTGTAGTCTTAACAGTAAACTATGCCGACTAGGGATCGGGCCACGTTAATTTCTGACTGGCTCGGCACCTTACGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGTGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTGATAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGATAACGAACGAGACCTTAACCTGCTAAATAGCCAGGCCGGCTTTGGCTGGTCGTCGGCTTCTAGAGGGACGTCGGCGTTTAGCCGACGGAAGTTTGAGGCAATAACA
4	KJR 27	Pigeon (*Columba livia*) dropping	*Naganishia albida* (*C. albidus*)	MT 102624	>MT102624.1 Naganishia albida isolate KJR27 small subunit ribosomal RNA gene, partial sequence AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACTTCAGGCCCGACGGGGTGGTCTGCCTCACGGTATGTACTATCCGGTTGGGCCTTACCTCCTGGTGAGCCCGTATGTCGTTTACTCGGTGTGCGGGGGAACCAGGAATTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCATATGCCCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTCTAGGATCGCCGTAATGATTAATAGGGACGGTTGGGGGCATTAGTATTCAGTTGCTAGAGGTGAAATTCTTAGATTTACTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGACGTTTTCATTAATCAAGAACGAAGGTTAGGGGATCAAAAATGATTAGATACCGTTGTAGTCCTAACAGTAAACTATGCCGACTAGGGATCGGGCCATGTTCAACTTTTGACTGGCTCGGCACCTTACAAAAAATCAAATTCTTTGGGTTCTGGGGGAAGTATGGTCGCAAGGCTGAACCTTAAAAGAATTGACGGAGGGGCACCACCAGGCTTGAAGCCGGCGGCTTATTTTAACTCATCACGGGGAAACTCACCAGGTCCGACACATACTAGGATTGACACATTGATATTTCTTTCTTGATTCTCTGGGTGGCGGTGCATGCCCGTTCTAAGTTGGTGGAGTGATTTGTCTGGTTAATGCCCAATCACGAACGTACATCTTAACCTGCTAACTACACCGATCGGGCTTTGAGCTGCACCCTCTATTCTTTA