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RESEARCH ARTICLE

Acoustic variations in the geographically isolated populations of L punctulata in India.

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Manuscript Info	Abstract
	Birds use verity of acoustic signals for their daily communication. These signals are very important for their daily life in individual recognition, food hunting, prey alarm etc. For successful reproduction acoustic signals are very important in mate recognition and attraction. However signals are very much affected by local habitat and geographical pressures and well reflected in populations and genetic structure. In present work geographical variation in the calls of *L punctulata* has been investigated. Results indicate that *L punctulata* is mono-syllabic in morning calls. Spectrographically no variations were observed across all locations. However in parametric comparisons variations were observed across all geographical regions. Population level acoustic signatures were seen in majority of individuals. UPGMA tree based on the parametric distance indicates population level structure in both notes with some admixture. This indicates the possibility of local level difference in genetic structure of *L punctulata*.

Introduction:-

In avian communication birds use a variety of acoustic signals to modulate their vocalization. These acoustic signals may be long and complex or short and simple, and may occur in particular rhythm. The pattern of vocalization can grant insight into the behavior and ecology of birds. Further these patterns can also provide better understating of geographical variation or impact of geography on avian vocalization (Mundinger, 1982; Beecher & Brenowitz, 2005; Podos & Warren, 2007). Several studies revealed that in oscine songbirds vocal dialects or vocal characteristics are commonly shared among the groups of individuals with some noticeable changes in the context to individuals of same species of the nearby or distantly far located individuals groups (Marler & Tamura, 1962; Mundinger, 1982). These geographical specific changes in vocalization mainly arise in the process of song learning (oscine Passeriformes, Psittaciformes, and Trochilidae; Mundinger, 1982; Podos & Warren, 2007). In contrary in most of the species of suboscines and non passerines birds vocalization is understood to be innate and under the influence of genetics (Mundinger, 1982; Zink & Remsen, 1986). Vocalization studies on non passerines exhibit that their calls can vary over distantly far located geographical scales with their varying genetical makeup (Isler et al., 2005; Nyari, 2007).

In oscine passerine birds vocalization is more subject to continuous learning rather than innate. Offspring's learned songs and calls from their parents and neighbors and therefore it is greatly under the influence of biological and cultural evolution (Slater, 1989; Beecher & Brenowitz, 2005). Many species of oscine birds shows significant variation in vocal dialects over their geographical distribution ranges. It appears often in form of local dialect which exists only over short geographical range (Marler & Tamura, 1962; Baker & Cunningham, 1985).

Across large geographical distance most oscine bird species show significant regional variation in vocalization that is often recognized to their learning and the accomplishment of local dialects through successful sexual selection (Mundinger, 1982; Podos & Warren, 2007). However few oscines species show very little variation in vocal dialects over vast geographical distances (Martens, 1996). Vocal similarities across geographical ranges are to be projected if vocal functions primarily act as a signal for species recognition. However such acoustic signals could also be shaped if all individuals hold just few common acoustic characteristics (Hunter, 1980; Price, 2008). This link between vocal similarity and variability across geographical range can provide an excellent opportunity to understand the role of local adaptation in framing vocal dialects in widely distributed species.

Lonchura is a genus of the estrildid finch family, which includes munias mannikins, and silverbills. They are resident breeding birds in Africa, Asia, Australia and in Sunda Islands. Munias are prominent member of the genus are widespread throughout the Afrotropical, Oriental, Australian and Melanesian regions. They range from West Africa to the Arabian Peninsula and through Asia to China, southwards to New Guinea and Australia. In India Lonchura have 5 species and 12 subspecies are present (Ali, 2001). The species *Lonchura punctulata* is widely distributed across the geographical scale from peninsular tips to Himalayan foothills and from Western coast to North Eastern part of India. *L punctulata* is common crop pest and easily available near paddy fields. *L punctulata* is opportunistic and monsoon breeders (Chandola et al., 1975; Chandola-Saklani et al., 1983). It times their breeding cycle according to circannual clock and season but it breeding times differ across the longitudinal ranges (Chandola-Saklani et al., 1983). In southern part of India it breeds earlier than compare to northern part of India. This suggested the geographical variation among the individual groups. In the present work we test whether the vocal call structure of *L punctulata* changes across the geographical ranges sharply or it changes gradually over geographical scales.

Materials and Methods:-

L punctulata is a widely distributed resident species in India. Recordings were made on four different geographical regions across the scale. Recoding site are Bijnor (Himalayan Foot Hills), Lucknow (Gangetic Plain), Hyderabad (South Central India), Thrisur (South India, Western Ghat) (Fig 1). All recording were made in morning sessions between the months of Feb to May. All recordings were carried out in natural habitat of birds. Recordings were made using TASCAM HDP2 digital sound recorder with wireless plug on transmitter SKP-500G2, Sennheiser. Unidirectional ME-66 microphones with K-6 power module Sennheiser were used, to avoid undesirable sound, and to improve the sound quality.

25-30 individuals were recorded from each site. Total 120 individual recordings were made. To avoid repetition of same individuals after every recording site were changes consistently. The recording was made from 10-20 meter distance from roosting site to avoid disturbance to birds. Normally birds come out of roosting site in groups of 2-5 or individually and park themselves within proximity. The unidirectional recording mic was pointed towards the individual bird for fine recording.

Recording was transferred to computer at 16bit, 22.05 kHz sampling rate. Data were analyzed in AviSoft SAS-Lab Pro (Specht, 2012). Each call screened manually for identification of syllable and notes. The noise above 1000 Hz falling between two syllables was not considered for study. Analysis was performed at two levels: Qualitative (visual/spectrogram) and Quantitative (temporal/spectrum /parametric).
Sonograms were prepared with 256 point transform and Hamming analysis window setting. The corresponding frequency resolution and bandwidth were 86 and 112 Hz respectively. Overlap was set at 50% with the temporal resolution 5.8 ms and 1/bandwidth 9.4 ms.

Sonograms were analyzed visually to identify the syllable and notes by subject similarity contrast method (Lei et al., 2005). Further analysis was done by multivariate analysis on note duration (DUR), frequency at start of note(PFS), frequency at end of note (PFE), frequency at centre of note (PFC), peak frequency (PFMAX), minimum frequency (MINFMX), and maximum frequency (MAXFMX) (Khanna et al., 1997, Lei et al., 2005). These parameters together constitute the structure/behavior of notes which in turn form the composite behavior of syllable/phrase/song. These are individual and species specific also and thus, may differ from individual to individual and population to population. A bird can detect these changes to identify whether their neighbors are from same population or from different population.

Data were further tested for normality by K-S test. The test of homogeneity of variance was also performed to assess the homogeneity of data. Normal distributed data were further subjected to multiple comparisons through ANOVA.

The unnormal data were analyzed through non parametric method (Kruskal-Wallis test). Data were analyzed with SPSS 15.0. A correlation matrix was constructed through correlation algorithm implemented in AviSoft Correlator program.

Result:-

Total 120 individuals were screened for individual geographical level variation. The syllable was found to be composed of two notes. The structure of first note was short and quick, second note was flat and longer. Measurements were taken for both the notes of syllable separately. In some individuals syllable was found to end with two more notes, third and fourth. These two additional notes were also short. These notes were not included for study because they were present only in few individuals (<8).

Spectrographically we did not observe any difference in syllable structure across the populations. We find the similar structure in all population. This would suggest that *L punctulata* might be mono syllabic in the basis of morning call. However we observe significant differences parametrically in both notes.

First note:-

The duration of first note varies from 0.012-0.023 ms. The frequency range is from 1491-5524Hz. The highest peak frequency at start of note (PFS) was found in Bijnor population (Bij,2765±62), followed by Hyderabad (Hyd, 2353±107) and Kerala (Klp, 2346±160). The peak frequency end of note (PFE) was observed maximum in Lucknow (Lko, 2942±291), followed by Hyd (2878±94). The peak frequency of centre of notes (PFC) were maximum in Bij (2933±61), followed by Lko (2872±456). The peak frequency (PFMAX) was observed maximum in Bij (3131 ±61) and lowest in Klp population (2685±172). The minimum frequency (MIMFMAX) was observed in Bij (1491±33). The maximum frequency (MAXFMX) 5524±179 observed in Klp, followed by 5323±69 in Hyd population.

K-S test for normal distribution and test of homogeneity of variance suggested that parameter PFC, PFMX, MINFMX and MAXFMX are not normally distributed, thus they were subjected to non parametric test (Kruskal-Wallis test). The DUR, PFS and PFE are normally distributed, thus the multivariate test (ANOVA) could be applied to them.

The Kruskal-Wallis test suggested, there was a statistically significant difference between the different populations in PFC, PFMAX, and MAXFMX (Chi-Square=7.951, 7.195, 52.94, P<0.05). Mean rank varies from 31-69 for Hyd, 22-48 for Lko, 23-69 for Klp and 45-50 for Bij population. The multivariate analysis (ANOVA) was used for DUR, PFS, and PFE. ANOVA results indicate the DUR is significantly different in Hyd–Bij population, while other population from Hyd is not different. Lko did not vary significantly from others except Bij in DUR. The DUR was not observed significantly different between Klp and other populations and between Bij and others. In parameter PFS, Hyd population are significantly different from Lko and Bij except Klp. Lko and Klp populations are significantly different from Bij population but not with others. Parameter PFE did not vary significantly in any all population. These both tests (Kruskal-Wallis & ANOVA) indicate that the populations are significantly different from each others.

The correlator matrix and Euclidean similarity matrix tree of NJ algorithm and simple cluster indicate there is no defined cluster, although there are several nodes which were population specific. Node A consists of population Hyd and Lko together. Node B primarily of Lko population. In node C Hyd population was represented chiefly. In node D Bij population with Klp were present mostly (fig2). PCA analysis also supports this structure, where individuals from different populations formed cluster together. To check the individual assignment CVA analysis were carried out. This observed 49% of individuals can be rearranged into other group.

These results suggest that there are intra-population variations and these are reflected at inter- level also. In other words, intra-population variations together make populations specific character which leads to inter population variations.

Second note:-

The duration of second note was 0.064-0.835 ms. The frequency range varies from 2661-4552 HZ. The highest PFS value was found in Hyd population (3442±42), followed by Bij (3262±23) and Klp (3208±53). The PFE observed maximum in Hyd (3520±20), followed by Klp (3208±53). The PFC were maximum in Hyd (3579±24), followed by Bij (3435±16). The PFMAX was observed 3571±25, maximum in Hyd and 3368±23 lowest in Lko population.

MINFMX was observed in Klp (2395±96). The MAXFMX 4552±68 observed in Lko, followed by 4520±24 in Hyd population. K-S test for normal distribution and test of homogeneity of variance suggested that parameter DUR, PFS and MAXFMX are not normally distributed, thus they are subjected to non parametric test (Kruskal-Wallis test). The normally distributed PFE, PFC, PFMAX and MINFMAX were subjected to multivariate test (ANOVA).

The Kruskal-Wallis test suggested, there was a statistically significant difference between the different population on DUR, PFS and MAXFMX (Chi-Square=25.8, 32.57, 54.53 with P<0.0001 respectively). Mean rank varies from 61-73 for Hyd, 26-33 for Lko, 42-73 for Klp and 48-60 for Bij population. The multivariate analysis (ANOVA) indicates the PFE is significantly different between Hyd-Lko and Hyd-Bij population. The Lko population was significantly different from all other populations on PFE. The populations Klp-Lko, Bij-Hyd and Bij- Lko were also significantly different from each other. In PFC parameter Hyd is significantly different from all other populations. Lko was also significantly different from Hyd and Bij. Klp was not different from others except Hyd. Bij was different from Hyd and Lko but not with Hyd. In parameter PFMAX Hyd population was significantly different from all other populations. Klp, Lko and Bij were only different form Hyd population in PFMAX, In MINFMX all the populations were significantly different from each other except Klp-Bij population. PCA analysis also supports this structure, where individuals from different populations formed cluster together.

In cluster analysis on correlation matrix and Euclidean distance, different types of clusters were observed among populations. It was observed that some nodes group together individuals from different populations. Node A and B consists of populations of Lko, with some individuals of Hyd and Bij. Node C consists of population Hyd. In node individuals of Bij populations are cluster together, also some individuals of Lko populations cluster in this node. In node E individuals of Hyd populations clustered.

Node F consists of individuals from all the populations. It was also observed that individuals of one population from different population clusters with other populations (fig 3). PCA analysis also supports this structure, where individuals from different populations formed cluster together. CVA classifiers detect 35% of individuals can be rearranged into other group. These results indicate that inter population variations are prevalent enough to distinguish them as different dialect regions. Also while inter population variations are present in some individuals inter-individuals variation are enough to categorize them as different populations.

Discussion:-
Bird vocalization is not only species specific but also individual specific. This individual specificity helps them to identify within population. Based on morning call *L punctulata* would be suggested as mono syllabic. Although some other syllables were also present, they were detected only in few individuals. The qualitative comparison suggested, there are no variations among the populations. But quantitative comparisons of PFC, PFMAX, MAXFMX and MINFMX suggested populations are significantly different from each other. Thus the syllable is common and present in all populations in sonogram quality but population specific when judged by quantitative parameters. This syllable can be treated as homologous. It must be cautioned that in acoustic communication, spectrum (temporal/frequency) shapes the structure of signal. The small variation in the signal can easily detect. However, these small variations are not evident visually (spectrographically). Birds can easily detect the temporal/frequency modulations, and discriminate inter- and intra species variations.

If a species is widely distributed, some geographical differences in syllabic pattern might be reflected. This difference is most obvious in species where repertoire size is small- one or few. Such as in white-crowned sparrow Baptista (1975) observed few birds of this species sing more than one song type, and song of all birds in a particular area are similar to one another but in a geographical range this difference became more distinguishable. However they all shared a common pool of syllables, buzz, trill and whistle was introduced as geographical representation. In *L punctulata* spectrographically, no variations occurs in within and among population. Although there is a significant difference between the populations in parametric methods. The possible reason for parametric variation may be habitat matching and genetic adaptation. Hahn et al., (2016) explained if a species occurs in a variety of different habitats, geographical variation in its song might stem from the process of acoustic adaptation.

Despite wide distribution of *L punctulata* from 10^0 N to 30^0 N on the Indian subcontinent vocal structure does not vary much perhaps because of similar ecology. In white-crowned sparrow Handford (1981) found less variation over short distances, when distances increase difference become clearer. This difference was only in the start or end of syllable in trill form. However these varied from habitat to habitat. There is an inverse relationship between the

frequency and latitude, the higher the altitude the lower the frequency. In *L punctulata* parametric variation were observed in similar habitat. The northern and southern populations were found to be diverse from each other. It indicates the geographical variation with same habitat may not influence the vocal structure (spectrograph) so much, but they affect the vocal quality. Handford & Lougheed (1991) also made similar observation in rufous-collared sparrow.

The parametric methods suggested that populations are far distant from each other. This population specific structure was also observed in cluster diagrams. We observed clear lineage specific pattern. This lineage specificity was also supported by our Mt DNA sequences (12S rRNA & 16S rRNA, Unpublished) genetic studies. The northern and southern populations are distinct from each other forming two big dialect regions. Some admixture can also be seen in both vocal and genetic studies. This may be due to local migrations. Also the clustering tree indicates similar topology in both cases e.g in distance based NJ tree the population specific cluster was observed. However, the population structure is more discrete in vocal parameters. Similar pattern was observed by MacDougall-Shackleton & MacDougall-Shackleton (2001) in Orinatha. He also found a correlation between genetic and song difference but it was not sturdy. It would suggest low gene flow, in the absence of barriers between dialects regions. In rufous-collared sparrow, Lougheed & Handford (1992) found that dialect variation was of no value in predicting genetic variation among populations, nor is variation in mitochondrial DNA related to song variation. However there studies on song phrases level not reflecting notes the fundamental unit. Our results relate the genetic & notes first time here. The studies of Wright & Wilkinson (2001) on yellow-naped amazon parrot suggest there is a clear dialect in contact call, but a high degree of gene flow between dialect areas. Our studies on *L. punctulata* morning call suggested clear dialect regions among the sampling populations. The dialect area size correlate with latitude and the extent that the population migrates, which may in turn relate to differences in experience and in dispersal (Irwin et al., 2008). However it remains possible that current dialect boundaries do restrict dispersal, but dialect origins are too recent for sequence variation to have accumulated. It remains to be understood how these variations in notes impact the syllable/song/phrase and their sociobiology.
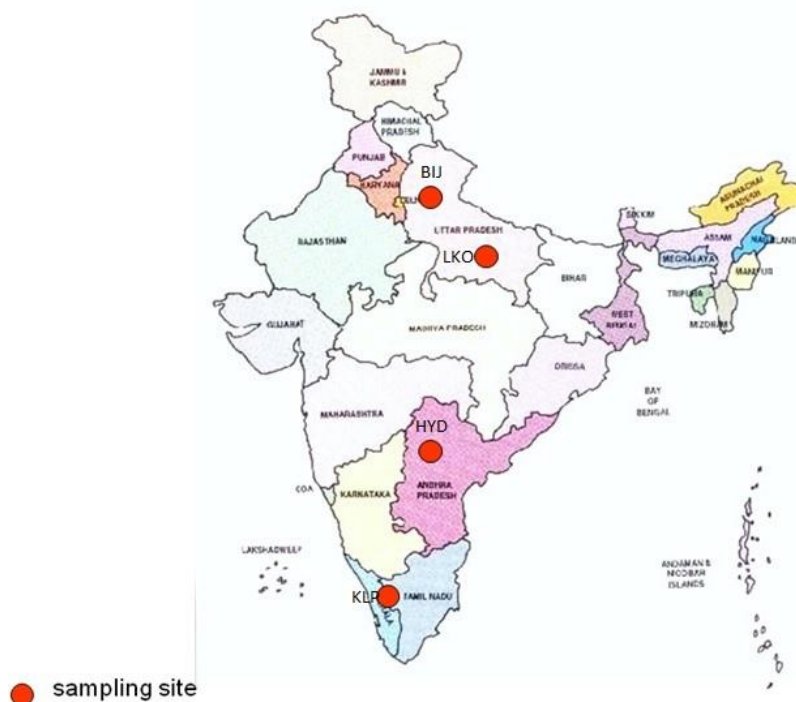
Acknowledgement:-

Fig 1:- Sampling Location in India. BIJ: Bijnor, LKO: Lucknow, HYD: Hyderabad, KLP: Kerala.
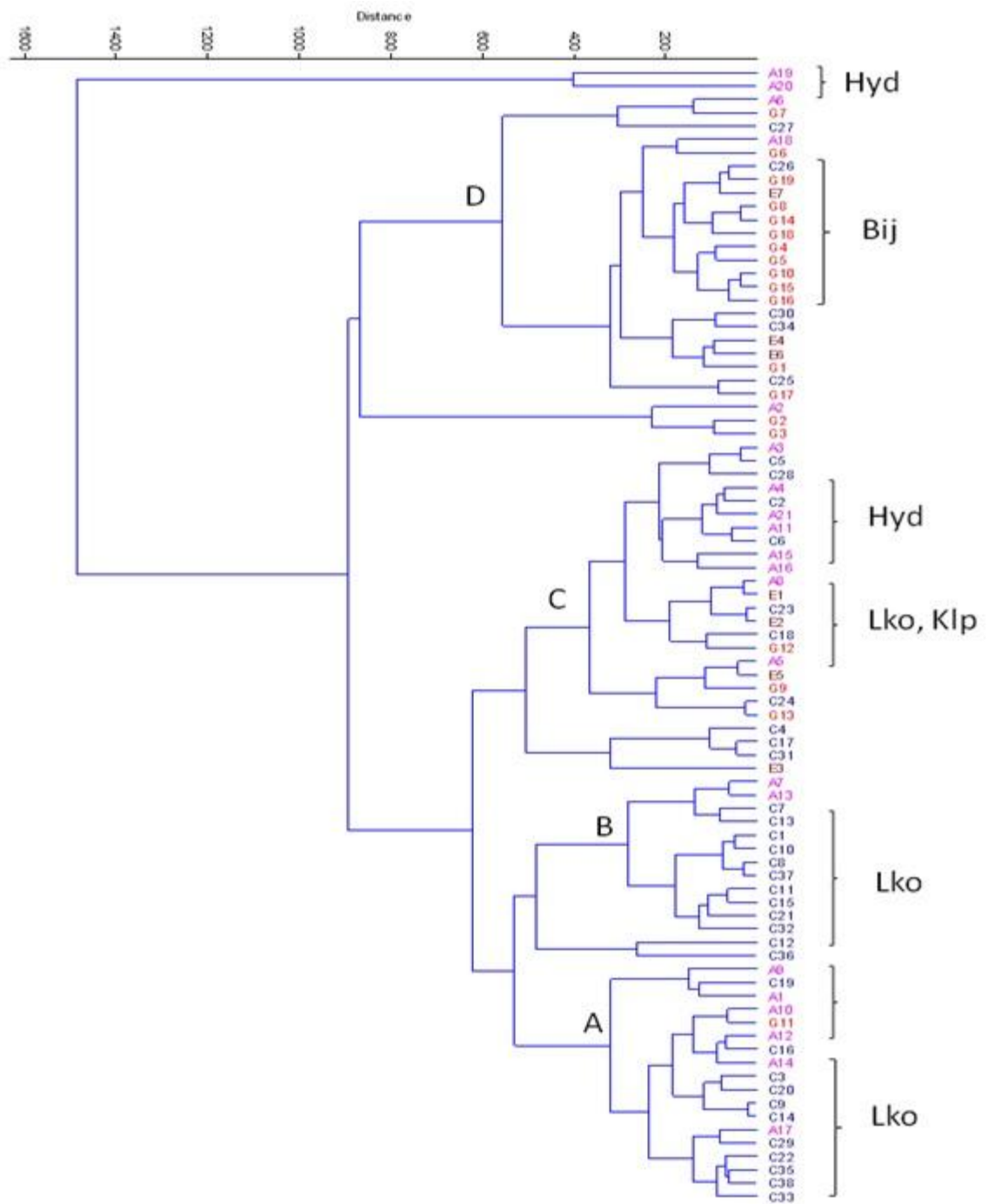
Fig 2:- NJ tree based on Euclidean distance similarity matrix on parametric comparisons for note 01. Population specific cluster can be observed easily but some intermixed clusters were also presents.
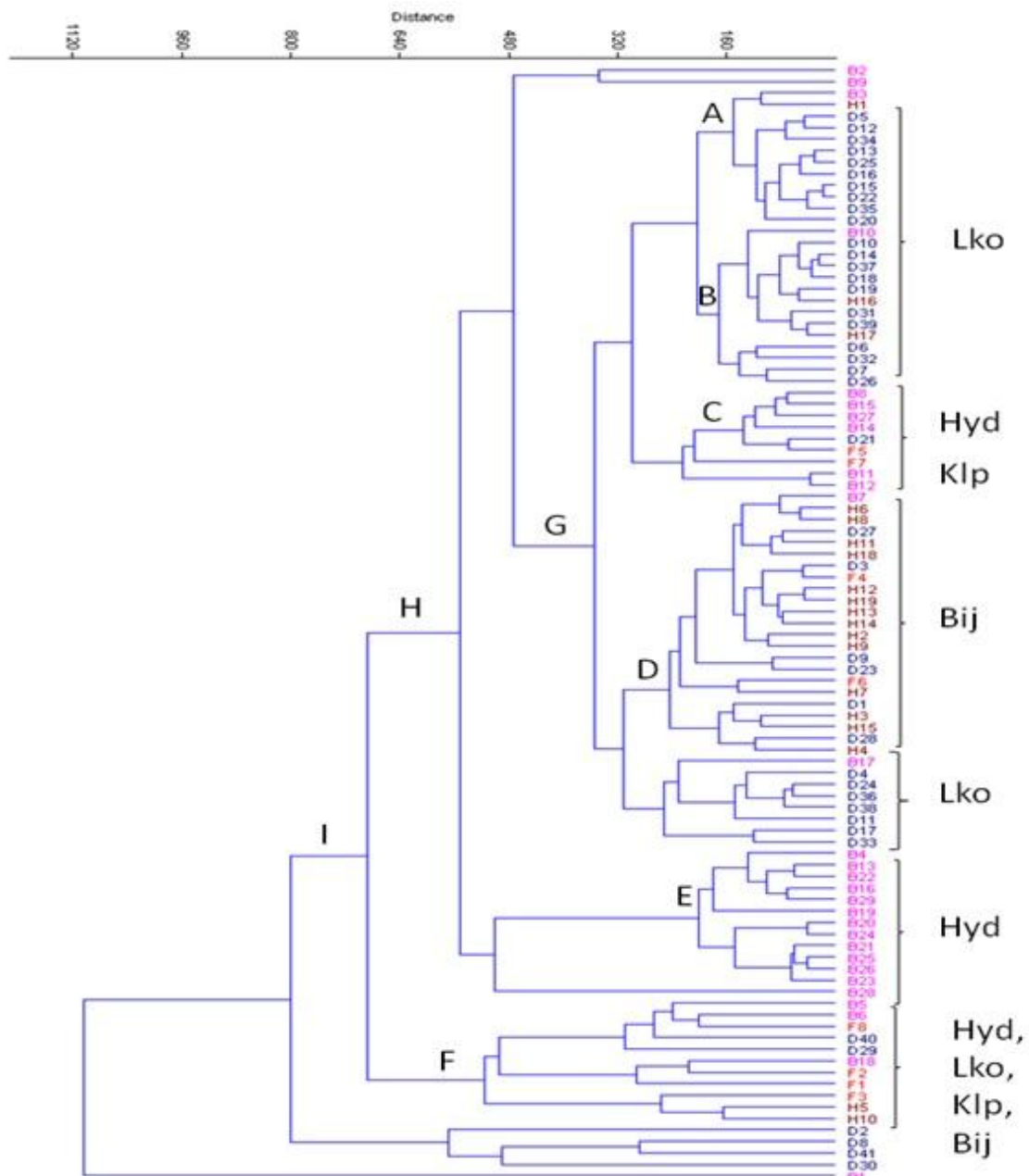
Fig: 3:- NJ tree based on Euclidean distance similarity matrix on note 02. Pollution specific clusters are more distinct here in comparison to note 1.

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