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Multifractal detrended cross-correlation analysis of coding and non-coding DNA sequences through chaos-game representation

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HIGHLIGHTS

- We propose a new approach combining CGR and 2D MF-X-DFA methods to analyze DNA sequences of unequal lengths.
- The multifractal characteristics of coding and non-coding sequence of eight prokaryotes are studied.
- The existence of strong multifractal behavior is observed in the nucleotide sequences.

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ABSTRACT

We propose a new approach combining the chaos game representation and the two dimensional multifractal detrended cross correlation analysis methods to examine multifractal behavior in power law cross correlation between any pair of nucleotide sequences of unequal lengths. In this work, we analyzed the characteristic behavior of coding and non-coding DNA sequences of eight prokaryotes. The results show the presence of strong multifractal nature between coding and non-coding sequences of all data sets. We found that this integrative approach helps us to consider complete DNA sequences for characterization, and further it may be useful for classification, clustering, identification of class affiliation of nucleotide sequences etc. with high precision.

1. Introduction

In a recent, various studies have been carried out to understand the characteristics of genomic sequences which are too long and complex in nature. In 1990, Jeffrey proposed a method to visualize primary DNA sequence structure using Chaos Game Representation (CGR) [1]. It provides the structure of DNA sequence of any length including entire genome in a compact two dimensional plot (image) which possess different geometric patterns such as parallel lines, triangles, squares and even some patterns show a fractal geometrical structure. Deschavanne and his coworkers in their study provided a link between CGRs and genomic signatures [2]. This motivated the researchers to apply CGR analysis on DNA sequences to classify

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sequence segments, phylogenetic analysis, secondary structure of the genome, protein sequences etc. [3–8]. More recently, J.J. Han and coworker have carried out the multifractal analysis of DNA sequence integrating the chaos game representation and wavelet transform modulus maxima method [9].

Similarly, a large number of studies have been carried out in developing different methodologies to characterize the correlation behavior and multifractal nature of a non-stationary time series. Until now many researchers have contributed their work using the methods like R/S analysis, structure function, detrended fluctuation analysis, wavelet transform modulus maxima, average wavelet coefficient method, multifractal detrended fluctuation analysis, detrended moving average method, wavelet based fluctuation analysis methods etc. For analyzing correlation behavior and fractal nature [10–17]. These methods found applications in various fields ranging from finance, technological, biological and physical sciences [18–40]. Some of the above mentioned methods have contributed a significant role in DNA sequence analysis [9,12,13,15]. Also some of the one dimensional multifractal analysis methods were extended to analyze higher dimensional data sets [41–45].

Recently, it was reported that there exists cross-correlation behavior in simultaneously recorded data sets. Podobnik and coworkers have developed a new approach namely detrended cross correlation analysis (DCCA) through which the cross correlation behavior of any two non-stationary time series can be investigated [46,47]. Later, Zhou proposed a generalization of DCCA called multifractal detrended cross correlation analysis (MF-X-DFA) to characterize cross-correlation behavior and multifractal nature of two cross-correlated time series [48]. Jiang and his coworkers have also developed a method called multifractal detrended moving average cross correlation analysis to study the cross correlation between two time series [49]. Using these above mentioned approaches the cross correlation behavior and multifractal characteristics studies have been carried out in analyzing time series of physiology, financial, and natural sciences [50–59]. In the further research, most of these one-dimensional time series methods on long range correlation and cross-correlation analysis have been extended to study higher dimensional data sets [41,43,45].

Our main goal of this work is to study the multifractal cross-correlation behavior of coding and non-coding DNA sequences whose lengths are not equal in size. Earlier studies on cross-correlation analysis between coding and non-coding sequences were carried out using 1D MF-X-DFA, in which they have considered only a portion of DNA sequences with equal lengths [59]. This procedure disregards the remaining portion of data to make the data with equal lengths to perform cross-correlation analysis which may lead to biased results. In such case, a more appropriate approach is required to characterize the cross-correlation behavior of the DNA sequences which are of unequal lengths.

In this paper, we propose a new approach combining the chaos game representation (CGR) theory and 2D multifractal detrended cross-correlation analysis (MF-X-DFA) method to analyze any pair of nucleotide sequences whose lengths are not equal in size. As a case study, we have analyzed the cross-correlation between the coding and non-coding DNA sequences of some prokaryotes. Section 2 describes about CGR and MF-X-DFA procedure and Section 3 shares the result and discussion. The conclusions to the study is given in Section 4.

2. Methodology

2.1. CGR of DNA sequences

The CGR is an algorithm which uses an iterative mapping technique to visualize the DNA sequences as an image that reveals patterns of different structure. The DNA sequence is consists of four nucleotides A (adenine), G (guanine), T (thymine), and C (cytosine) and then the CGR image is confined to unit square. Let us consider the length of the DNA sequences is L and the vertices of the unit square is labeled by the nucleotide A(0, 0), T(1, 0), G(1, 1), C(0, 1). The position of each nucleotide can be calculated by the iterative function system

$$X_i = 0.5(X_{i-1} + G_{ix}) \tag{1}$$

$$Y_i = 0.5(Y_{i-1} + G_{iy}). \tag{2}$$

Here X_i and Y_i are the co-ordinates of the ith nucleotide and the position of the each nucleotide is calculated from the half of the previous position but for the first nucleotide X_{i-1} and Y_{i-1} are given by the center of the square (0.5, 0.5). G_{ix} and G_{iy} represent the co-ordinates of the vertex of the each nucleotide. By repeating the above procedure the co-ordinates for all nucleotides in a DNA sequence can be calculated and then plotted as CGR image which reveals different patterns.

2.2. Two dimensional MF-X-DFA method

The MF-X-DFA method was developed by W.X. Zhou to unveil the multifractal nature and cross-correlation behavior between any one dimensional and two dimensional data sets [47]. In our work, we make use of 2D MF-X-DFA method to analyze the CGR images. The following steps are the procedure of 2D MF-X-DFA method.

Step 1: Consider any two surfaces (images) $x(i, j)$ and $y(i, j)$ of identical sizes, where $i = 1, 2, \ldots, M$ and $j = 1, 2, \ldots, N$.

Step 2: Then divide the surfaces into $M_s \times N_s$ disjoint square segments of the equal size i.e. $s \times s$, where $M_s = M/s$ and $N_s = N/s$. Each segment of the surfaces is denoted by $x_{v,w}$ or $y_{v,w}$ such that $x_{v,w}(i, j) = x(l_v + i, l_w + j)$ and $y_{v,w}(i, j) = y(l_v + i, l_w + j)$ for $1 \le i, j \le s$, where $l_v = (v - 1)s$ and $l_w = (w - 1)s$.

Step 3: Each segment $x_{v,w}$ or $y_{v,w}$ is calculated as follows:

$$X_{v,w}(i,j) = \sum_{k_1=1}^{i}\sum_{k_2=1}^{j} x_{v,w}(k_1,k_2) \quad \text{and} \quad Y_{v,w}(i,j) = \sum_{k_1=1}^{i}\sum_{k_2=1}^{j} y_{v,w}(k_1,k_2)$$

here $1 \le i,j \le s$.
Step 4: Now the detrended covariance of any two segments is calculated as:

$$F_{v,w}(s) = \frac{1}{s^2}\sum_{i=1}^{s}\sum_{j=1}^{s}\left[X_{v,w}(i,j) - \tilde{X}_{v,w}(i,j)\right]\left[Y_{v,w}(i,j) - \tilde{Y}_{v,w}(i,j)\right] \tag{3}$$

where $\tilde{X}_{v,w}$ and $\tilde{Y}_{v,w}$ are the local polynomial trends of $X_{v,w}$ and $Y_{v,w}$ respectively. The trend function is chosen as the simplest plane $\tilde{u}(i,j) = ai + bj + c$ which is adopted for our analysis.
Step 5: The qth order fluctuation function of detrended cross-correlation $F_{xy}(q,s)$ is obtained by squaring and averaging over all segments,

$$F_{xy}(q,s) = \left(\frac{1}{M_s N_s}\sum_{v=1}^{M_s}\sum_{w=1}^{N_s}\left[F_{v,w}(s)\right]^{q/2}\right)^{1/q}. \tag{4}$$

According to l'Hospital's rule when $q = 0$, we have the fluctuation function as

$$F_{xy}(q,s) = \exp\left(\frac{1}{2M_s N_s}\sum_{v=1}^{M_s}\sum_{w=1}^{N_s}\ln\left[F_{v,w}(s)\right]\right). \tag{5}$$

Here 'q' is the order of the moment that can take any real value.
Step 6: The steps 2–5 is repeated for variable scale size 's' for different values of q. The power law behavior of the data is obtained by analyzing the fluctuation function.

$$F_{xy}(q,s) \sim s^{h_{xy}(q)}. \tag{6}$$

As is known if the cross correlated surfaces show monofractal behavior then the scaling exponents $h_{xy}(q)$ values behave independent of q values. For the multifractal behavior the $h_{xy}(q)$ values depend on q values. If the two data set are same i.e. $x = y$ then this 2D MF-X-DFA is same as 2D MFDFA. Further for positive q, $h_{xy}(q)$ describes the scaling behavior of the segments with large fluctuations. On the contrary, for negative q, $h_{xy}(q)$ describes the scaling behavior of the segments with small fluctuations.

The multifractal behavior of the cross-correlated data sets can also be studied by evaluating the $f_{xy}(\alpha)$ spectrum. The Legendre transform of $\tau_{xy}(q)$ gives values of $f_{xy}(\alpha)$:

$$f_{xy}(\alpha) \equiv q\alpha_{xy} - \tau_{xy}(q). \tag{7}$$

Here $\tau_{xy}(q) = qh_{xy}(q) - D_f$, for the 2D CGR images in our study we consider $D_f = 2$. Also the values of α_{xy} is obtained from $\alpha_{xy} = \mathrm{d}\tau_{xy}(q)/\mathrm{d}q$. The strength of the multifractality can be calculated from the width of the $f_{xy}(\alpha)$ spectrum. Broader the spectrum stronger the multifractality and the narrower spectrum depicts weak multifractal behavior.

3. Results and discussion

In our study, we consider the genomic sequences of eight prokaryotes in which two are from Archaea and six from Bacteria. The data was obtained from the EMBL-EBI database (http://www.ncbi.nlm.nih.gov/). The detailed information about the phylum, species and strain are given in Table 1. We have separated the coding sequences (CS) and non-coding sequences (NCS) using MATLAB programming and found the lengths of the CS and NCS are unequal in size. The obtained CGRs of CS and NCS in their present form are not suitable to perform computational analysis because it is merely a graphical representation. So, we have used the mathematical representation of CGR (i.e. frequency chaos game representation (FCGR) which is a numerical matrix) to perform the 2D MF-X-DFA analysis. To obtain the FCGR we have divided the CGRs into $2^k \times 2^k$ grids and each square grid is considered as an element in the FCGR matrix. The total number of points present inside each square grid is used as matrix element which is nothing but the number of occurrences of each length k oligonucleotide present in the sequence. In our analysis, we have used $k = 10$, so that we get the FCGR matrix with size 1024×1024. To perform the 2D MF-X-DFA analysis, the size of the CGR images should be at least 8×8 (i.e. $k >= 3$) such that the minimum scale's' will be 1/4th size of the CGR image (i.e. $s = 2^1$). The major advantage of the Chaos Game Representation method is that it can represent any length of a DNA sequence including entire genomes in a form of an image. This approach helps one to make an image of equal size of coding and non-coding DNA sequences even though the lengths of sequences are not in equal size. We have applied the 2D multifractal detrended cross correlation analysis method on the calculated FCGR matrices of CS and NCS sequences of all eight data sets. For illustration purpose, the CGR images of the coding and non-coding DNA sequences of *Aeropyrum pernix* are shown in Fig. 1.

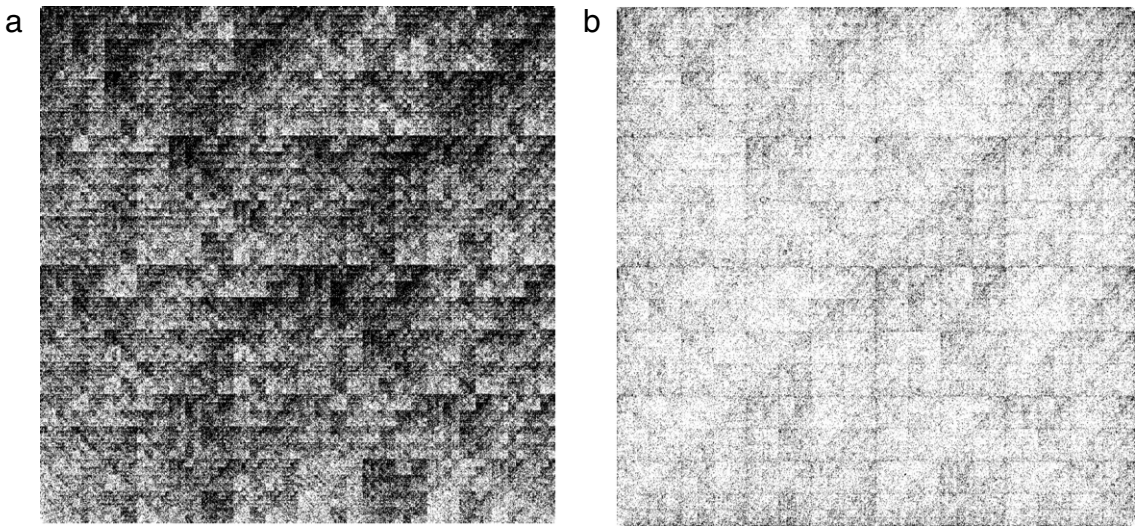

Fig. 1. CGR images of *Aeropyrum pernix* (a) coding sequences (b) non-coding sequences.

Table 1
List of eight prokaryotes considered for analysis of which two are archaea and six bacteria.

Domain	Phylum	Species	Strain	Short name	Length of data
Archaea	Crenarchaeota	*Aeropyrum pernix*	*Aeropyrum pernix* K1 uid57757	Apernix	1 669 696
Archaea	Euryarchaeota	*Archaeoglobus fulgidus*	*Archaeoglobus fulgidus*	Afulgi	2 178 400
Bacteria	Actinobacteria	*Mycobacterium tuberculosis*	*Mycobacterium tuberculosis* H37 Rv uid57777	MTb	4 411 532
Bacteria	Spirochetes	*Borrelia burgdorferi*	*Borrelia burgdorferi* CA382 ui d214794	Borrelia	910 736
Bacteria	Proteobacteria	*Haemophilus influenza*	*Haemophilus influenza* KR494 ui d219323	Hinflu	1 856 176
Bacteria	Proteobacteria	*Bacillus subtilis*	*Bacillus subtilis* PY79 uid229877	Bsubtil	4 033 459
Bacteria	Hyperthermophilic	*Thermotoga maritima*	*Thermotoga maritima*	Tmarit	1 869 644
Bacteria	Hyperthermophilic	*Aquifex aeolicus*	*Aquifex aeolicus*	Aquifex	1 551 335

From our analysis, we observe that the fluctuation function, $F_q(s)$ increases linearly as the size of the scale s increases for all values of q varying from -10 to 10 with step size 0.2 showing existence of power law behavior. The cross-correlation analysis was performed between CS–CS, CS–NCS, NCS–NCS sequences respectively. From the calculated scaling exponents, multifractality nature is evident as we observe that the $h_{xx}(q)$, $h_{yy}(q)$ and $h_{xy}(q)$ values for all data sets depend on q values. In this work, the values of $h_{xx}(q)$ represents the CS–CS analysis, $h_{yy}(q)$ represent the NCS–NCS analysis, and $h_{xy}(q)$ represent the scaling exponents of cross-correlation between CS and NCS data sets. The calculated $h(q)$ values for all the data sets, the $h(q)$ values decreasing with increasing q values and this is evident for multifractal nature. This is clearly shown in Fig. 2. We also observed from the calculated $h(q)$ values of CS–CS and NCS–NCS analysis, for negative q values the $h_{xx(yy)}(q)$ values (i.e. small fluctuations) of CS sequences are greater than NCS sequences for all the data. Similarly for large fluctuations i.e. positive q values, the $h_{xx(yy)}(q)$ values of CS are greater than that of NCS except for the subjects *A. Pernix*, and *Mycobacterium Tuberculosis* where $h_{xx(yy)}(q)$ values of NCS sequences are greater than the CS sequences. From the results of cross-correlation analysis between CS–NCS, one can observe that $h_{xy}(q)$ values of large fluctuations (i.e. negative q values) are lying between the CS–CS and NCS–NCS correlation analysis. We also generated the singularity spectrum for all the data sets and the resulted broader spectrum indicates the existence of strong multifractality nature and this is evident from Fig. 3. It is also observed from the multifractal spectrum that for the species *Bacillus subtilis*, *Haemophilus influenza*, and *M. tuberculosis*, the $f_{xy}(\alpha)$ spectrum lies in between the $f_{xx}(\alpha)$ and $f_{yy}(\alpha)$ spectrum, where as in all other species we find existence of cross over.

It is worth emphasizing Cristina stan and co-workers has carried out a study on similarity analysis between two DNA sequences using chaos game representation method [60]. In their work, they set a condition for partitioning the cell, if the value of k is too large then many of the FCGR matrix elements may have zeros, to avoid such situation the maximum value of k can be calculated by making use of the formula given below;

$$k_{\max} = \text{int}\left(\frac{\ln N}{2\ln 2} - 1\right) \tag{8}$$

where N is the total length of the sequence. We have carefully analyzed the data using above formula for fixing the suitable 'k' values and also for different 'k' values such as 7, 8, 9 & 10. We found that there is no significant change in the results of $h(q)$ and $f(\alpha)$ spectrum. This emphasizes that there will not be any difference in the results if one chooses arbitrary 'k' values. But the above mentioned formula may be useful to reduce the computation time for very large data.

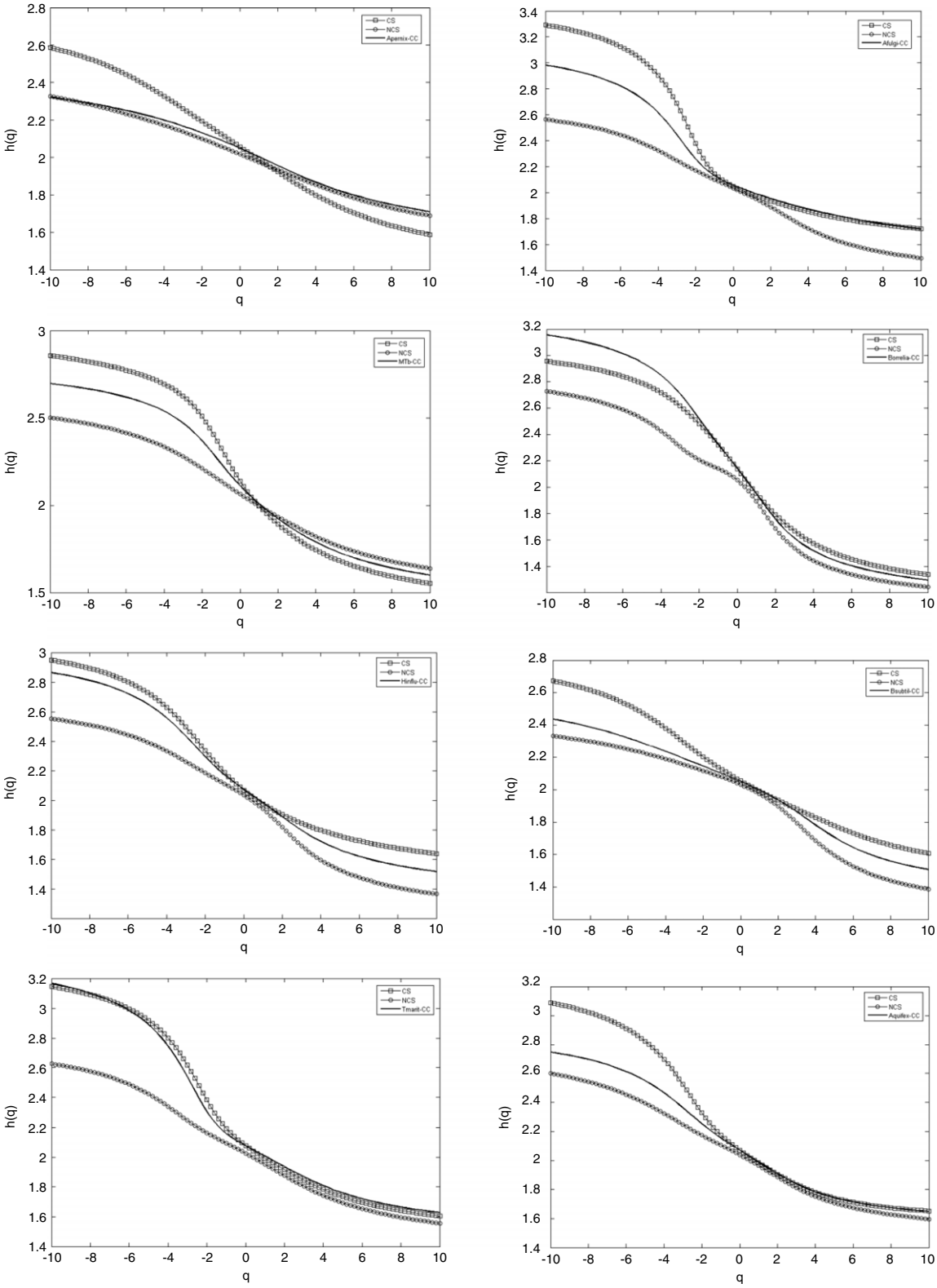

Fig. 2. The scaling exponent values of $h_{xx}(q)$, $h_{yy}(q)$, $h_{xy}(q)$ for different q values show non-linear behavior, which implies the presence of multifractal behavior for all bivariate time series.

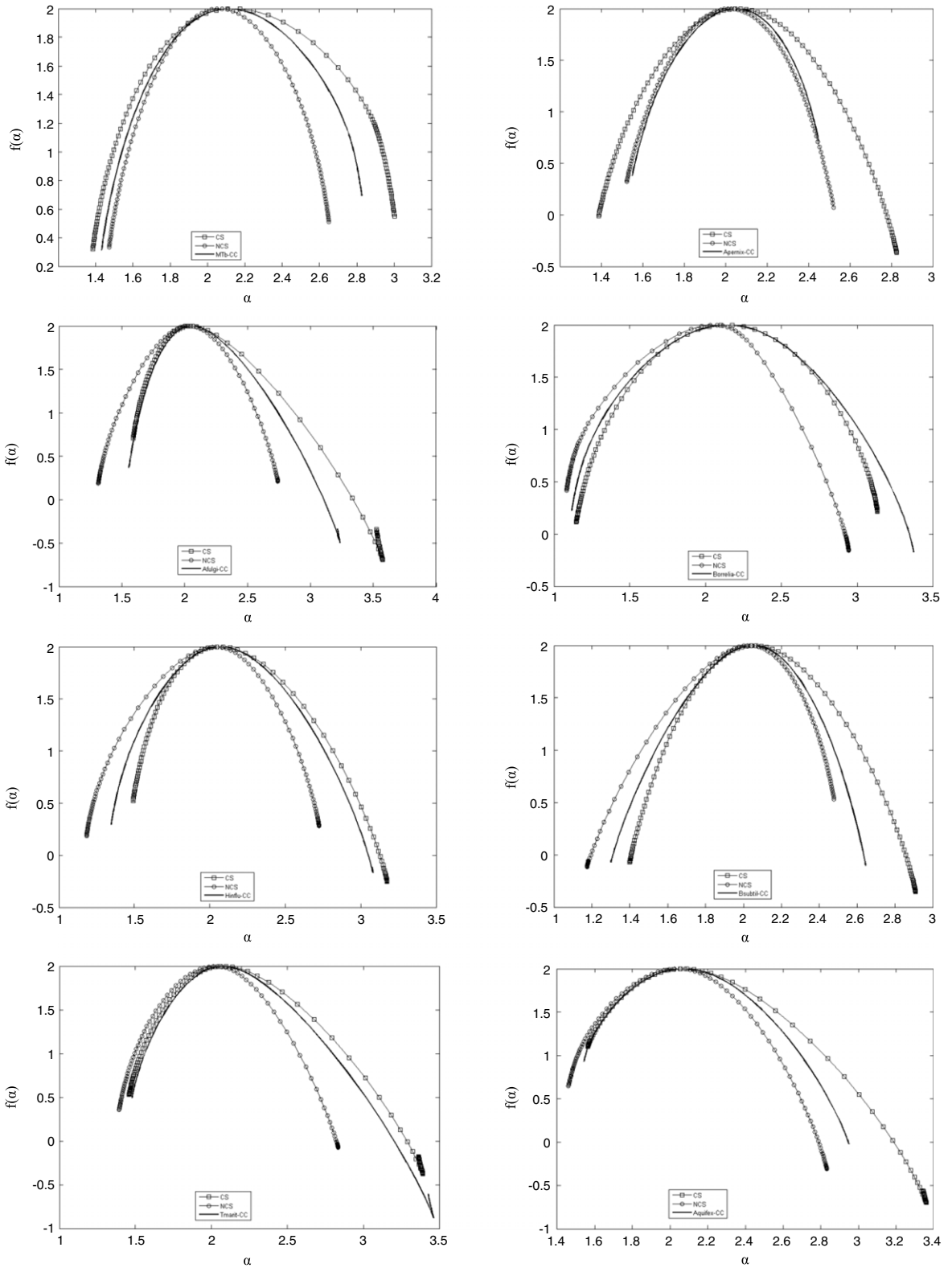

Fig. 3. The $f(\alpha)$ spectrum shows the strength of multifractality of all bivariate time series is shown through. The broad spectrum implies strong multifractality and narrower spectrum indicates the weak multifractal behavior.

From this multifractal analysis, we found that CGR images play a vital role in converting the DNA sequences into images that provide statistical information. Apart from this it also helps to construct the CGR images of equal sizes even if the length of nucleotide sequences of different species are unequal to perform the 2D MF-X-DFA analysis. Consideration of full length of DNA sequences for cross-correlation analysis may provide results with high accuracy when compared to analysis on 1D MF-X-DFA by chopping the nucleotide sequences to have equal lengths for analysis.

4. Conclusion

In conclusion, we have presented a new approach combining CGR and 2D MF-X-DFA and have applied this method to study the multifractal cross-correlations of coding and non-coding DNA sequences of eight prokaryotes. We also found CGR is an efficient tool that helps in considering the unequal lengths of DNA sequences for multifractal cross-correlation analysis and this is evident from our study. We suggest this integrative approach may find useful in studies like classification, clustering, identification of class affiliation of nucleotide sequences, protein sequence etc.

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