Cluster Analysis asa Methodology Within Phylogenetic Systematics to Construct Phylogenetic Trees

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I.   INTRODUCTION

All evolutionary studies of groups of species are based on the choice of appropriate characteristics for rebuilding their phylogenies (a phylogeny is the relationship or kinship among species in general and tries to reconstruct evolutionary relationships). A phylogenetic analysis reconstructs the evolutionary relationships between species, which descend from common ancestors and, furthermore, whichare the genetic distances or separation times between these species [1].

To generate a phylogenetic analysis characters must have two requirements: independent of each other and be homologous, they have the same origin and the same function in all organisms Study

The nature of those characters can be varied. Any source of validated and proved phylogenetic information can provide characters for an evolutionary study. Among the main evolutionary studies that have been developed stand two methods: The methods that have been taken as morphological characters base in which the presence of physical characteristics that describe the species is identified, and methods that have been based on molecular characteristics as the sequence DNA[2]. These characters are recorded in a data matrix within which, the state in which the character has been observed is represented with zero if it is absent or one if present respectively, and whether it is a character that may be present in the species with different values (multi-state) within the data matrix can be represented by the value corresponding to that character [2].

For this reason, homologous characters, once they have been validated and proven, may be taken as the basis for an evolutionary study because they provide enough information for the reconstruction of a phylogenetic tree.

II.   DATA MATRIX

The species to be analyzed are defined based on the each scientist interested group. Therefore, once the data set has been obtained, it needs to be translated into a structure that allows fully represent the relationships with each other.

For this, it is common to find the data represented by a matrix in which taxa (species) are grouped in rows and the characters in columns.

An important component to know is the term external group (out group) whose main function is that it can be used as a comparison group on which we could take it as a base to do the math measurement and comparison to join groups each other and to entrench the resulting cladogram. If we do not include the out group within our data matrix the cladogram will lack the root. The matrix (1) shows an example of a data set containing the group out.

Matrix 1. Example of a data matrix (Own creation)

Matriz de Datos				
	1	*2*	*3*	*4*
Out Group	0	0	0	0
Taxon 1	0	0	1	0
Taxon 2	1	0	0	1
Taxon 3	1	1	0	0
Taxon 4	1	1	0	1

Matrix (2) presents real data, in which a set of 10 taxa is shown and each taxon has 21 features.

Matrix 2. Real Data Matrix [3]

1	Pachymerus	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
2	Kytharinus	1	1	1	1	0	1	0	0	1	1	0	0	1	1	2	1	1	0	0	1
3	Megacerus	1	1	1	1	1	1	0	0	0	1	1	0	0	1	1	0	1	0	1	2
4	Callosobruchus	1	1	1	1	1	1	0	1	0	1	0	1	0	2	1	0	1	1	2	1
5	Conicobruchus	1	1	1	1	1	1	1	0	0	1	0	0	0	1	1	1	0	1	2	1
6	Rhipibruchus	1	1	1	1	1	1	0	1	0	1	0	1	0	1	1	0	1	1	1	1
7	Pectinibruchus	1	1	1	1	1	1	0	1	1	1	0	0	0	1	1	1	1	1	1	1
8	atrolineatus	1	1	1	1	1	1	0	1	0	1	0	1	0	1	1	0	1	1	2	1
9	walker	1	1	1	1	1	1	0	1	0	1	0	1	0	1	1	0	1	1	2	1
10	lumae	1	1	1	1	1	1	0	1	0	1	0	1	0	1	1	0	1	1	2	1

III.   CLADOGRAMS

A cladogram is a diagram of data as a tree reflecting the genealogical relationships of terminal taxa [4]. Phylogenetic trees or cladograms could be rooted or not. The Rooted trees have a particular node called root from which begins to come off the evolutionary path that is formed. A tree not rooted specifies the relationships among taxa but does not define the evolutionary path see Fig. (1)

Figure1 Root tree and not root tree [5]

Based on the number of taxa to be used in the study will be a wide variety of trees. For example, if we talk about 3 species A, B, C. there may be three rooted trees and one without roots see Fig(2).

(A,(B,C))   ((A,B),C)   (B,(A,C))

Figure2. Tree with three taxon [6]

According to [2] the possible number of rooted trees for n taxa can be calculated from,(1):

$$N_r = \frac{(2n-3)!}{2^{n-2}(n-2)!} \quad \text{Para n>2}$$

Equation1 Calculate root trees

Where:

Nr is the number of rooted trees.
 n is the number of taxa.

And the number of unrooted trees can be calculated by, (2):

$$Nu = \frac{(2n-5)!}{2^{n-3}(n-3)!}$$

Equation2 Calculate unrooted trees

Where:
Nu is the number of trees without roots.
n is the number of taxa used.

The number of possible trees rooted with n taxa is equal to the number of trees unrooted for n-1 taxa, the number of trees increases as n increases. Thus, from 12 species becomes difficult to quantify the number of trees with and without root that could be obtained (because it is an intractable problem since the compute all possible trees has a very high computational cost). For example, a year has 31,536,000 seconds, a Pentium IV processor executes four million instructions per second, which runs about $126\ 144x10^9$ instructions per year. Assuming a tree in each instruction is performed, and leaning in Figure 3, for 20 species it would take 65 011 380 years to show all the trees and for 30 species will take $3.925x10^{25}$ years [6].

Thus, when n is large, the expert can´t analyze all the trees generated, as only one of those trees correctly represents the true evolutionary relationship. Therefore, heuristics that can generate the correct trees are used.

Especies	Número de árboles
1	1
2	1
3	3
4	15
5	105
6	945
7	10.395
8	135.135
9	2.027.025
10	34.459.425
11	654.729.075
12	13.749.310.575
13	316.234.143.225
14	7.905.853.580.625
15	213.458.046.676.875
16	6.190.283.353.629.375
17	191.898.783.962.510.625
18	6.332.659.870.762.850.625
19	221.643.095.476.699.771.875
20	8.200.794.532.637.891.559.375
30	$4.9518x10^{38}$
40	$1.00985x10^{57}$
50	$2.75292x10^{76}$

Figure 3 Number of trees depending the number of species[6]

IV.   Methods for constructing phylogenetic trees.

Once we have determined the group of species and created the data matrix, we can start to construct phylogenetic trees through different methods, however all this, it is necessary to say that you can build many trees and each of these will constitute a different evolutionary hypothesis [7].

A part to consider into cladistics is that it is not an intuitive system, but is based on empirical methods of reconstructing using strict rules for example the common ancestors linked through synapomorphies. For this reason [5] classifies empirical methods used to reconstruct phylogenies follows:

Based on Distances
Algorithmic way:
- ultra-metric (UPGMA Unweighted Pair Group Method With Arithmetic mean)
- Additives (NeighborJoining)
- Optimization form
- The relationship between neighbors (Neighborliness)
- Distances transformed

Based on characters:

- By optimization criteria:
- Hennig
- Maximum Likelihood (ML = Maximum Likelihood)
- Parsimony (Maximum Parsimony) Estimation of the goodness of reconstruction using analytical techniques and resampling (Bootstrap, Jacknife, Decay).

Phylogenetic systematics or cladistics was proposed by German entomologist Willi Hennig in 1950 to make phylogenies with a methodology that was testable and repeatable which, until that date could not be done. There was not a way to follow, instead of, the investigator´s experience said which groups were more similar each other [8]. Therefore, the proposed method will be compared with Hennig algorithm, since today several entomologist still rely with such method.

V.   HENNIG

Hennig's argument considers the information of each character one at a time. It is easy to understand by a small example taken from [9]. In this example we will take as a base a data matrix containing 4 study groups (taxa) and 6 characteristics (Matrix 3)

Matrix 3 Hennig Data Matrix [9]

	Characters				
	1	2	3	4	5
Outgroup	0	0	0	0	0
A	1	0	0	0	1
B	1	1	0	1	0
c	1	0	1	1	0

1. Character 1 unites the taxes (groups) A, B and C because they share the apomorphic characteristic 1 (Fig. 4).
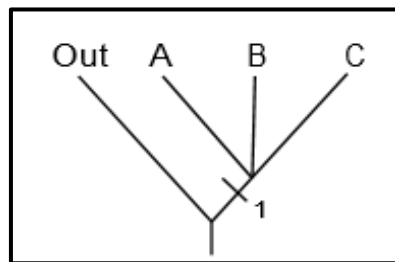
Figure3. Tree with character 1[9]

2. Character 2 - the derivative character is found only in the taxon B, and does not provide much information about the relationships between taxa (Fig. 5).
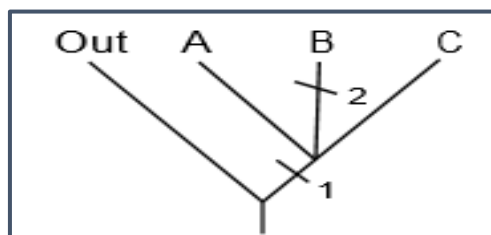
Figure4 Tree with character 2[9]

3. Character 3 - the derivative character is autopomorfic for group C (Fig. 6).

Figure5 Tree with character 4 [9]

4. Character 4 - The derivative character is synapomorphic and unites the taxa A and B (Fig. 7)

Figure6 Tree with character 4 [9]

5. Character 5 - The derivative character is an autopomorfic for the taxon A (see Figure 8)

Figure7 Tree with Character 5 [9]

The real data matrices are rarely so simple. However, the concept is the same. Within the following flow chart the function of our algorithm is shown (Fig. 9)

Figure8Hennig Flowchart

As seen in Figure 9, the programmed algorithm requires three cycles. Each cycle contains nested two cycles to tour the matrix $(A (n^3) + B (n^3) + C (n^3))$. Therefore the complexity is $O (n^3)$.

VI.   CLUSTER

Cluster Analysis is a multivariate statistical technique that seeks to separate or link elements or variables trying to achieve maximum homogeneity in each group and the biggest difference between the groups.

Cluster analysis has a tradition in many areas of research. However, the solutions obtained are not unique, far cluster membership for any number of solutions depends on many elements involved in the procedure chosen. Moreover, the cluster solution depends entirely on the variables used, the addition or destruction of relevant variables can have a substantial impact on the resulting solution. [10], classifies into two categories the conglomerates.

Partition Algorithms
Method of dividing the set of observations in k clusters, where k initially is set by the user.

Hierarchical Algorithms
They are methods that provide a hierarchy of divisions of a set of elements in n clusters, i.e., based on a study group can unite or divide such element in N conglomerates, the unions or divisions represent a hierarchical order in the final conglomerate. Therefore, hierarchical algorithms in turn can be agglomerative or dissociative (Fig 10).

- A dissociative hierarchical method follows the reverse direction, part of a large conglomerate and is dividing successive steps until each observation is in a different cluster.
- An agglomerative hierarchical method starts with a situation where each observation forms a conglomerate and successive steps are joining, until finally all situations are in a single cluster.

Jerárquicos

Aglomerativos
- Simple Linkage (Vecino más próximo)
- Complete Linkage (Vecino más lejano)
- Promedio entre Grupos
- Método del Centroide
- Método de la Mediana
- Método de Ward

Disociativos
- Linkage Simple
- Linkage Completo
- Promedio entre Grupos
- Método del Centroide
- Método de la Mediana
- Método de Ward
- Análisis de Asociación
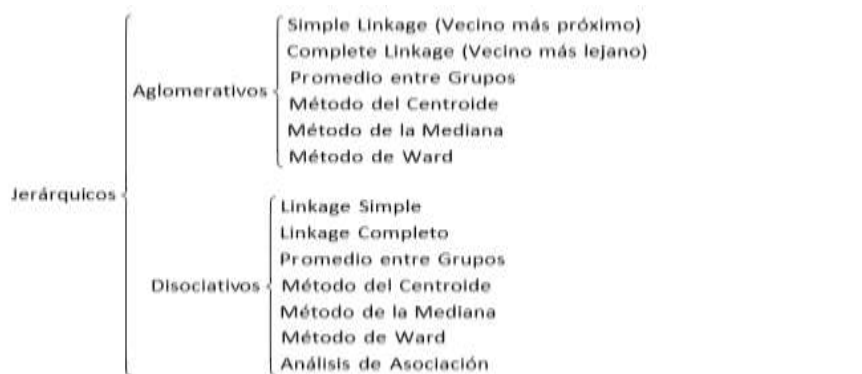
Figure9   *Hierarchical*
classification algorithms [10]

To bind variables or individuals we needed to have some numerical measures that characterize the relationships between the variables or individuals. Each measure reflects a partnership in a particular sense and is necessary to choose an appropriate measure depending on the problem being treated. The measures of association could be distance or similarity.

- When you choose a distance as a measure of association, the groups will contain similar individuals formed so that the distance between them must be small.
- When a similarity measure is chosen, the groups formed contain individuals with high similarity between them.

Stages of Cluster Analysis
Like any algorithm, you should identify the steps required for the analysis. The steps within the cluster analysis are:
1. Select variables
2. Choose the measures of association
3. Election of the cluster technique

Within the phylogenetic analysis presented below, the choice of variables has been determined by an expert who supports the accuracy of our data matrix. Our measure of association will be calculated based on the difference of values that have each of the homologous characteristics with respect to the set of taxa that form the data matrix. These differences will be calculated and represented in a distance matrix.As our goal is to form a single cluster from a set of elements, in each observation (iteration) has to form a conglomerate. This action is repeated until finally all taxa are connected in a single tree. For this reason the agglomerative hierarchical

algorithm Simple LinkAge was chosen (nearest neighbor) and will help us to determine the respective distance between other species belonging to the dataset was chosen to observe. This method allows us to create the tree in a simple manner. We need to create a matrix of distances in which the distances between species are marked respectively. Once said matrix calculated, we identify where there is less distance to join the species whose distance is less.

The distances between clusters are functions between observations, and therefore there are several ways to define them. We will use the minimum distance, also known as distance to the nearest neighbor (Fig. 11).
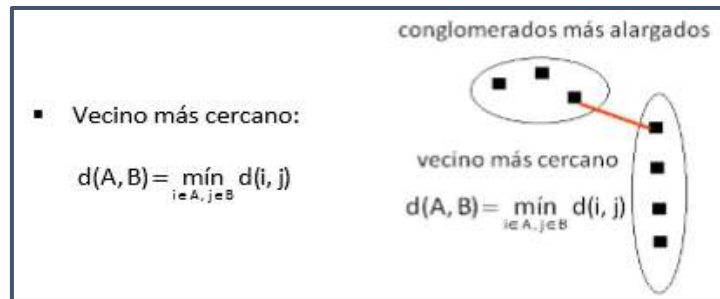
Figure10. Neighbor nearest distance[10]

Algorithm:
- Starts with a matrix with n taxa (data matrix) and a matrix n × n distances Δ = (δij) symmetrical with zeros on the diagonal.
- Groups with less distance between them (the two closest groups) is sought in the matrix of dissimilarities. Let U and V closest groups, and d (UV) its distance.
- U and V groups are joined, and the new group as (UV) is labeled. The dissimilarity matrix is updated as follows:
  a)   The rows and columns corresponding to the U groups and V are erased.
  b) One row and one column with the distances between the group (UV) and the remaining groups is added.
- Repeat steps 2 and 3, n - 1 times. In the end, all units will be included in a single group and labels have joined groups and distances with which joined (Hernandez, 2011). 11 shows the flowchart of the algorithm Conglomerates Simple LinkAge

For carrying out the second step, requires the definition of a measure of dissimilarity between groups. The dissimilarity measure that is defined determines the type of agglomerative method. The dissimilarities we will use is *the single Linkage* or *nearest neighbor*, in this method, the dissimilarity between two groups is difference among its closest members, i.e., if U and V are two groups, then defined as follows, (3):

$$duv = \min\{d_{ij} \ 0 : i \in U, \qquad j \in V\}$$

Equation 3 Simple Linkage dissimilarity measure
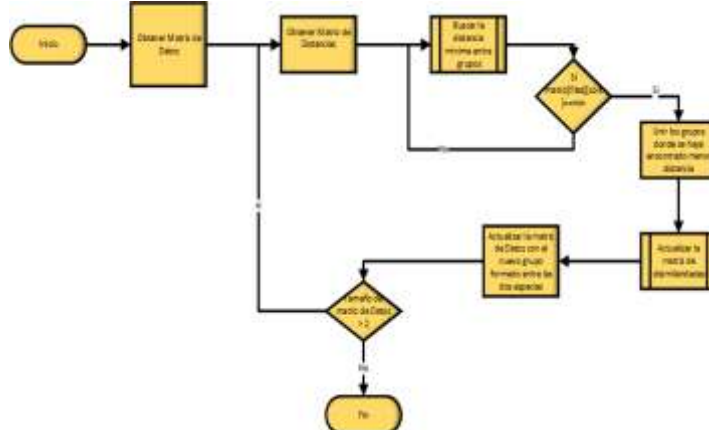
Figure 11 Conglomerate flowchart

As shown in Figure 12, there are two nested loops to find the minimum distance between groups and update the dissimilarities matrix (A (n^2) + B (n^2)), so that its complexity is $O(n^2)$. These two cycles are immersed in a cycle. So the algorithm has a $O(n^3)$ complexity.

To better understand how this algorithm works, see the following example: With our Data Matrix (Matrix 4).

Matrix 4 Data Matrix (own creation)

Taxón	Characters		
	1	2	3
A	1	1	1
B	0	1	1
C	0	1	0

The distance between each of the species is estimated. For calculations will have to observe the number of changes that have among taxa (Matrix 5)

Matrix 5. Matrix to calculate the distances between 2 groups (own creation)

A	1	1	1
B	0	1	1

The distance between A and B is only 1 because the first characteristic is one in the group A and 0 in group B. The characteristic 2 and 3 have the same value therefore does not increase the distance.
Successively, the calculations between all species in order to obtain the distance matrix (Matrix 6) are performed.

Matrix 6 Distances Matrix (own creation)

DistanceMatrix

Distancia	A	B	C
A	0		
B	1	0	
C	2	1	0

As we can see the distance matrix is a Symmetric matrix since d (A, B) = d (B, A). Therefore, we don´t need complete the matrix because the lower triangle of the matrix contain the same values as the upper triangle and diagonal of our distance matrix always be filled with zeros as d (A, A) = 0.
The minimum distance that we can find is between the taxon Band taxon C, therefore B and C form a new group (Fig. 13).

d (B,C)=1

Figure 12 Conglomerate (Own Creation)

Now the selected groups into the data matrixwill be modified as shown in the data matrix (Matrix 7)

Matrix 7 Data Matrix (Own Creation)

DistanceMatrix

Taxón	Characteristics		
	1	2	3
A	1	1	1
B-C	0	1	0

The new distance matrix is defined as follows: (Matrix 8):

Matrix 8 Distance Matrix (Own Creation)

DistanceMatrix		
Distancia	A	B-C
A	0	
B-C	2	0

d(A,B-C)=2

As can be seen, the distance matrices are calculated based on the data matrix and joins the group with its nearest neighbor (Fig. 14).
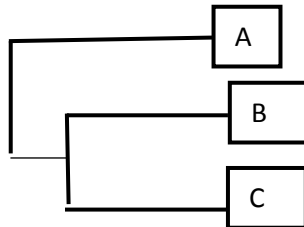

Figure 13 Conglomerate between A-B-C (Own Creation)

Creating a phylogenetic tree based on the cluster algorithm Linkage Simple
Matrix 9 is a data matrix taken from [3] which is used as a base for creating a cladogram using the Simple algorithm LinkAge

Matrix 9 Real Data Matrix [3]

From the matrix 9 proceed to calculate the first distance matrix. We must identify into the matrix which are the taxa that have less distance to each other (minor differences). An important factor to consider in the distance matrix data is that there may be different intersections between species containing the minimum distance 0 (zero). It is for this reason that the number of trees increases based on the number of taxa involved in the analysis as specified above (Matrix 10).

Matrix 10 Distance Matrix (Own Creation)

Matriz de Distancias

	1	2	3	4	5	6	7	8	9	10
1	0									
2	15	0								
3	11	6	0							
4	13	8	4	0						
5	12	7	4	5	0					
6	13	8	4	0	5	0				
7	14	5	5	3	4	3	0			
8	13	8	4	0	5	0	3	0		
9	13	8	4	0	5	0	3	0	0	
10	13	8	4	0	5	0	3	0	0	0
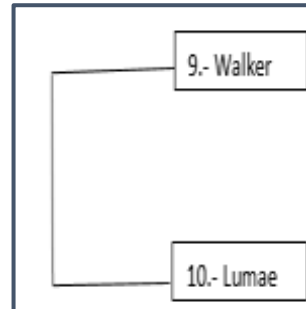
Figure 14 Conglomerate (Own Creation)

1. In the matrix 10 it can be seen that the groups have less distance each are the groups 910. Therefore these two groups have formed a new conglomerate that consists of the union of the two groups mentioned in Fig. 15 graphically describes that Union.
2. Recalculate the distance matrix (Matrix 11)

Matrix 11 Distance Matrix (Own Creation

Matriz de Distancias

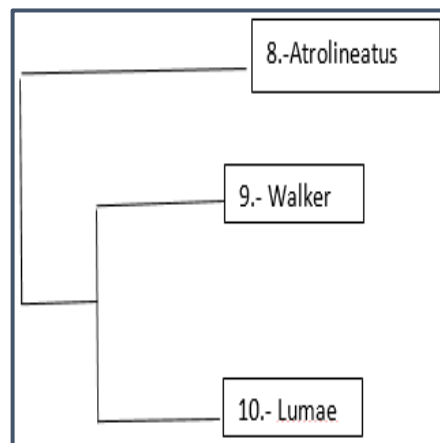|	1	2	3	4	5	6	7	8	910
1	0								
2	15	0							
3	11	6	0						
4	13	8	4	0					
5	12	7	4	5	0				
6	13	8	4	0	5	0			
7	14	5	5	3	4	3	0		
8	13	8	4	0	5	0	3	0	
910	13	8	4	0	5	0	3	0	0

Figure 15 Conglomerate (Own Creation)

The next element to be joined is given by the group 8. In the matrix 11 one can realize that effectively the groups sharing less distance are group 8 and group 910. Therefore, they must be attached to form the group 8910 (Fig. 16).
3. Recalculate the distance matrix (Matrix 12):

Matrix 12 Distance Matrix (Own Creation)

	1	2	3	4	5	6	7	8910
1	0							
2	15	0						
3	11	6	0					
4	13	8	4	0				
5	12	7	4	5	0			
6	13	8	4	0	5	0		
7	14	5	5	3	4	3	0	
8910	13	8	4	0	5	0	3	0
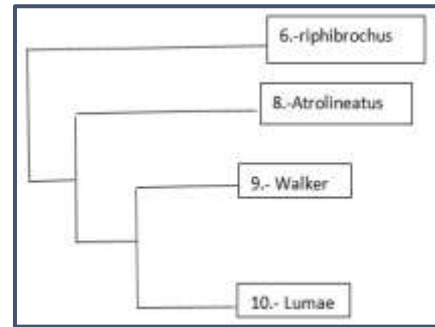
Figure 16 Conglomerate (Own Creation)

However, as we have seen, distance matrices must be calculated every time a new group is created. In the die 12 it can be seen that group 5 and intersection 8910 with the group are those with the minimum distance to each other. There are other intersections with different groups which could form new conglomerates. However, in our example the value of minimum intersection is found more in depth (see Figure 17) will be taken.

4. Recalculates the distance matrix obtained matrix 13:

Matrix 13 Distance Matrix (Own Creation)

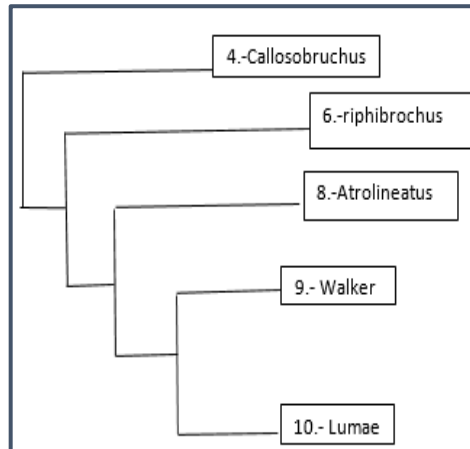|	1	2	3	4	5	7	68910
1	0						
2	15	0					
3	11	6	0				
4	13	8	4	0			
5	12	7	4	5	0		
7	14	5	5	3	4	0	
68910	13	8	4	0	5	3	0

Figure 17 Conglomerate (Own Creation)

The next group to form is given by the 4 and the group 68910, thus our new group will be called 468910 which are the groups that have been formed. This representation is reflected in cluster 4 (Fig. 18).

5. Recalculating the distances, the group 7 is added to the group 468 910 because in the distance matrix (Matrix 14) can be seen which are the groups in which there is less distance. The representation can be seen in Fig. 19.

Matrix 14 Distance Matrix (Own Creation)

	1	2	3	5	7	468910
1	0					
2	15	0				
3	11	6	0			
5	12	7	4	0		
7	14	5	5	4	0	
468910	13	8	4	5	3	0
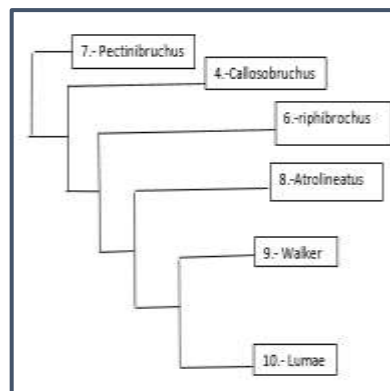
6. Proceed to do the calculations for the following matrix of distances resulting in the matrix

15.*Matrix 15 Distance Matrix (Own Creation)*

Matriz de Distancias

	1	2	3	5	7468910
1	0				
2	15	0			
3	11	6	0		
5	12	7	4	0	
7468910	11	7	3	4	0
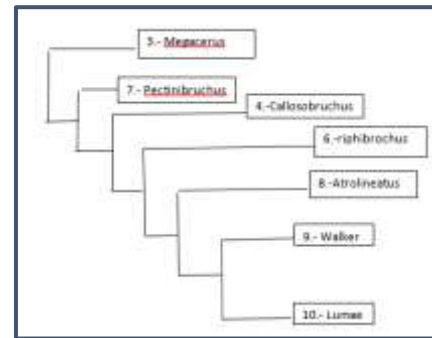
Figure 19Conglomerate (OwnCreation)

The new cluster is formed by joining the following groups with the shortest distance to each other. In the matrix of calculated distances (matrix 15) as in all previous distances matrices, we should calculate the distance matrix based on the data matrix. At this point to note that the matrix leaves the group 3 should join the conglomerate formed, their representation can be seen in the cluster (Fig. 20).

7. We calculate the distance matrix

Matrix 16 Distance Matrix (Own Creation)

Matriz de Distancias

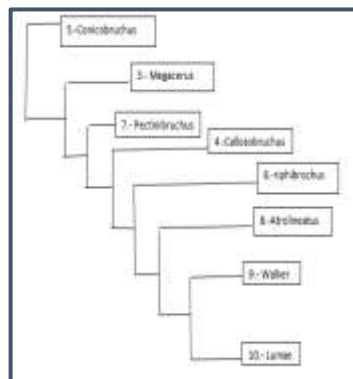|	1	2	5	37468910
1	0			
2	15	0		
5	12	7	0	
37468910	10	5	4	0

Figure 20Conglomerate (Own Creation)

The process is repeated for each iteration adding the lower groups apart. In this iteration group 5 joins the cluster as shown in Fig. 21.

8. In conclusion, the final matrix is calculated distances to finish taxa and form unite our overall conglomerate

Matrix 17 Distance Matrix (Own Creation)

Matriz de Distancias

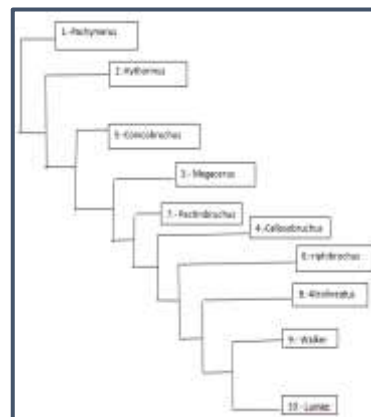|	1	2	537468910
1	0		
2	15	0	
537468910	9	6	0

Figure 21Conglomerate (Own Creation)

Our conglomerate to this point is almost complete. Up to this point we have the group which joins 537468910 with the Group 2 (Fig. 22).

As the distance matrix decreases, the graph increases, i.e., when a new cluster is created, we have the ability to identify which groups are those with minor differences and merge them together.When a taxon in comparation

with other taxa (both belonging to the study group) found the less distance, it is said that has found his nearest neighbor.

In Fig. 23a and 23b two cladograms based on the same data matrix are shown. You can see the similarity presenting the two cladograms each other. This is, the data matrix remains the basis for the process of creating the cladograma however, and these reconstruction methods are totally different.
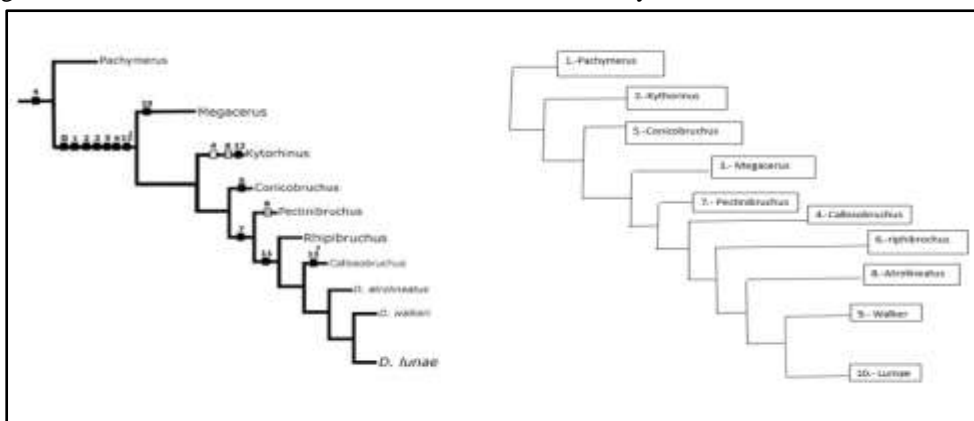

Figure 23a Phylogenetic Tree created with Hennig　　*Figure 23b Tree created with Clustering algorithm Simple LinkAge (Own Creation)*

VII. CONCLUSIONS

Phylogenetic analysis within entomology is a process that requires validation and work by the expert. Relations between taxa should be based on the similarities that have to take a phylogenetic tree that allows us to reconstruct the similarities between organisms.

The construction of phylogenetic trees or cladograms can be carried out by different methodologies that are based on probabilistic and mathematical principles which determine each of the different forms of reconstruction. For this reason, the use of clusters within the entomological field facilitates to the expert the phylogenetic analysis because can be used as a basis tool for generating genealogical relationships between species. It is convenient to say that the final results can be very similar to each other because are based on the original data matrix which tries to link the most essential features for analysis.

Cluster analysis uses many algorithms that can be used together to deliver different results, that is why its use within the economics, statistics and many disciplines their participation is essential for representing structured data [11].

There is no standardization in formulating cladograms since it depends on the methods used as mentioned above, also the data matrices may have different characteristics because of groups of study that are to be analyzed.

In computational part, as shown in Fig. 9 and 12, although the algorithmic complexity in both algorithms has an order $O(n^3)$ shows that the algorithm is simpler cluster for its understanding and programming.

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