CHAPTER 4


INTRODUCTION AND BRIEF HISTORICAL RESUME

Systematic and phylogenetic relationships in heterotrichid ciliates have not been critically investigated using modern taxonomic and phylogenetic methods. The work on the classification of these ciliates can be broadly divided into two periods. In the first period (upto late 1960s), much emphasis was laid on the gross morphological features such as shape and size of the body, macronuclear characteristics and also the shape and size of the peristome and infundibulum, whereas, the second period was (from early 1970s) dominated by the use of cortical features (argyrome and
infraciliature) to supplement the gross morphological characters. These served as the main basis for understanding and assessing the phylogenetic relationships of this group as a whole.

Heterotrichid ciliates are characterised by the presence of unique "système secants" (i.e. kinetal sutures) and these features have been recently used as taxonomic markers at generic level (Albaret, 1975). Besides these argentophilic structures recent workers (Albaret, 1975; Affa'a, 1979a & b, 1980, 1983 and 1986b) have also used other characteristics such as presence or absence of a sucker ("ventouse") on the inferior face of these ciliates together with the presence, shape and pattern of deposition of polysaccharide skeletal elements (especially those found associated with the sucker) in the classificational hierarchy of these ciliates. However, due to the challenging complexity, together with homoplasy of most of these morphological characteristics the phylogeny of these ciliates have been difficult to interpret. Parallelisms and reversals handicapped the earlier ciliatologists in their effort to produce a natural system of classification.

Albaret (1975) proposed a revised classification of endocommensal heterotrichid ciliates and also speculated their phylogenetic relationship. However, there is a pressing need for revising and reassessing the overall systematics
and phylogenetic relationships of these ciliates. This has been necessitated because: (1) numerous new taxa (genera as well as species) have been recently described (Affa'a, 1979, 1980 and 1986 b), some of which pose a problem regarding their taxonomic position, and (2) relatively new taxonomic and phylogenetic techniques have now become available which can be used for studying systematics of these ciliates. Earlier ciliate taxonomists used intuitive traditional (classical) approach in their evolutionary speculations and taxonomic procedure. For example, according to Corliss (1979) "recognition of presumed evolutionary trends ...... has long been practised by ciliatologists ...... although sometimes perhaps subconsciously." Consequently, the taxonomic decisions have been "a matter of personal conviction" based mostly on assigning unequal weights to different characters by different workers. Besides, numerous new genera which have been described recently (Affa's, 1978, 1979, 1980, 1981 and 1986 b) acquire a combination of characteristics that are intermediate between the currently recognized two families - Sicuophoridae (Amaro, 1972) Albaret, 1975, and Nyctotheridae Amaro, 1972. Same is true for some species which do not fit well in the genera in which they are placed.

Keeping the above facts in view the phylogenetic relationships and systematics of these ciliates is revisited and a new phylogenetic tree has been proposed.
to replace the "aging one" given earlier by Albaret (1975).

Two systematic approaches are currently being used by biologists. One of these, the numerical taxonomy (Sneath & Sokal, 1973) constructs phenetic classifications on the basis of overall similarity. These classifications do not always reflect phylogenetic relationships although some authors have used phenetic approach for making for making phylogenetic inferences (e.g., Moss, 1971; Bretsky, 1971; Findley, 1972; Jensen, 1975; Jensen & Eshbaugh, 1975 and Felsenstein, 1982). On the other hand, the cladistic taxonomy (Camin & Sokal, 1965; Henning, 1966; Farris, 1970, 1972, 1982 and 1983; Farris et al., 1970; Lundberg, 1972; Eldredge & Cracraft, 1980; Wagner 1980; Nelson & Platnick, 1981; Wiley, 1981; Felsenstein, 1983, Sober, 1983; Brooks et al., 1984; Hull, 1984; Janvier, 1984 and Humphries & Funk, 1984) constructs phylogenetic classifications which reflect genealogical descent. Phylogenetic classifications are more informative (all the characters are shown on the phylogenetic tree) and least ambiguous (Brooks et al., 1984).

Both pheneticiists and cladists attempt to make taxonomy logical, objective and repeatable. These methods according to Brinkhurst & Nemec (1987), "... force one to declare all the assumptions made, to define the character states of all the taxa and to avoid many errors in logic". The application of numerical taxonomy and cladistic techniques in protozoan taxonomy is already evident

MATERIALS AND METHODS

Twenty one taxa (O T U s = Operational taxonomic units) representing all the known genera to date belonging to the endocommensal heterotrichid ciliate families — Nyctotheridae Amaro, 1972 and Sicuophoridae (Amaro, 1972) Albaret, 1975 are included in the analysis (Table 33). This also includes representatives of three new genera recorded in the present investigation. The genus Sicuophora de Puytorac & Grain, 1968 is represented by three species, (1) S. heimi Albaret, 1970, (2) S. macropharyngae (Bezzenberger, 1903) Albaret, 1973, and (3) S. magn (Bezzenberger, 1904) Albaret, 1975 (= S. mabokensis Albaret, 1970) because this genus is probably not monophyletic. Genus Nyctotheroides Grassé,
Figure 68. Representing phylogenetic relationship and subclusters of the previously recognized families Sicuophoridae and Nyctotheridae (constructed according to single linkage clustering based on Jaccard's similarity matrix).
Figure 59. Representing phylogenetic relationship and subclusters of the previously recognized families Sicuophoridae and Nyctotheridae (constructed according to "UPGMA" algorithm based on Jaccard's similarity matrix).
TABLE 32. CHARACTERS AND CHARACTER-STATES CODED FOR HETEROTRICHID CILIATES ENDODOMENAL IN AMPHIBIANS AND REPTILES OF THE WORLD.

A. CHARACTERS:

1. Preoral kinetal suture formed by ventral and dorsal kineties at the anterior pole.
2. Kinetal suture at the apical end on the face inferior formed by the lateral kineties.
3. " dorsal and lateral kineties.
4. s dorsal and lateral kineties.
5. Superior lateral kineties.
6. inferior dorsal and lateral kineties.
7. Caudal dorsal and lateral kineties.
8. Dorsal and lateral kineties.
9. Ventral and dorsal kineties.
10. lateral kineties.
11. lateral kineties.
12. lateral kineties.
13. lateral and dorsal kineties.
15. Peristome originates near the apical end and is more or less longitudinally deposited.
16. The distal tip of the infundibulum coils upon itself and makes at least one complete turn.
17. Buccal orifice is more or less in the middle of the ventral side and not at the posterior pole.
18. Sucker reduced and is restricted towards anterior part of the inferior face.
19. Ventral longitudinal groove ("Gouttisre").
20. Dorsal

B. CHARACTER STATES:

For characters 1 to 13 & 18; State 'O' = Reduced or absent
State 'I' = Well developed

For character 14; State 'O' = Sucker absent or if present not armed with polysaccharide skeletal elements.
State 'I' = Sucker armed with polysaccharide skeletal elements.

For characters 15, 16, 17 and 21; State 'O' = Yes
State 'I' = No

For character 19 & 20; State 'O' = Absent
State 'I' = Present
<table>
<thead>
<tr>
<th>OTUs (TAXA)</th>
<th>CHARACTERS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 AD(I)</td>
</tr>
<tr>
<td>a) PRONYCTOTHERUS BOUCHERI Albaret et Mijne, 1975.</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>b) NYCTOTHERUS ALPHA Buhl, 1972.</td>
<td>1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2</td>
</tr>
<tr>
<td>c) NYCTOTHERIDAE N JUNEI Affa’s, 1979</td>
<td>1 0 1 0 0 1 0 1 0 0 0 0 0 0 0 0 0 2</td>
</tr>
<tr>
<td>d) NYCTOTHERIDAE ANOMALOUS Affa’s, 1979</td>
<td>1 0 1 0 0 1 0 1 0 0 0 0 0 0 0 0 0 4</td>
</tr>
<tr>
<td>e) NYCTOTHERIDAE HYLAN (Broussal, 1937) Albaret, 1975</td>
<td>0 0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 3</td>
</tr>
<tr>
<td>f) NYCTOTHERIDAE JUDEX Affa’s, 1980</td>
<td>0 0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 2</td>
</tr>
<tr>
<td>g) NYCTOTHERIDAE ARMOI Affa’s, 1980</td>
<td>0 0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 4</td>
</tr>
<tr>
<td>h) MULTNYCTOTHERUS COMMOTO (Tuzet, Manier et Jolivet, 1957) Albaret, 1970</td>
<td>1 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>i) PRONYCTOTHERUS DRAGOSCOAffa’s, 1983</td>
<td>1 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>j) PARASYCLOPHORA XAVIERAE Albaret, 1973</td>
<td>1 0 0 1 0 0 1 0 0 1 0 0 0 0 0 0 0 0 0 5</td>
</tr>
<tr>
<td>k) SICLOPHORA MACRAPHYRNGEA (Bezzenberger, 1903) Albaret, 1973</td>
<td>1 0 0 1 0 0 1 0 1 1 0 0 0 1 0 1 0 0 0 7</td>
</tr>
<tr>
<td>l) SICLOPHORA MAGNA (Bezzenberger, 1904) Albaret, 1975</td>
<td>1 0 0 1 0 0 0 0 1 0 0 0 0 1 0 0 0 0 0 4</td>
</tr>
<tr>
<td>m) SICLOPHORA NEIMEI Albaret, 1970</td>
<td>1 0 0 1 0 0 1 0 1 0 0 0 0 1 0 1 0 0 0 6</td>
</tr>
<tr>
<td>n) SICLOPHIDAE BASIGOLIS de Fuytorac et Oktam, 1967</td>
<td>1 1 0 0 0 0 0 1 1 0 0 0 0 1 0 1 0 0 0 6</td>
</tr>
<tr>
<td>o) SICLOPHORIDAE VARIABILIS N. GEN., N. SP.</td>
<td>1 0 0 1 0 0 0 0 1 0 0 0 0 1 0 1 0 0 0 5</td>
</tr>
<tr>
<td>p) METASICLLOPHORA FEITZI Albaret, 1973</td>
<td>1 0 0 0 0 0 0 0 1 1 0 1 0 1 0 1 0 0 0 7</td>
</tr>
<tr>
<td>q) NEOCLOPHORA BENIFORMIS N. GEN., N. SP.</td>
<td>1 0 0 1 0 0 0 0 1 0 0 0 1 0 1 0 1 0 0 6</td>
</tr>
<tr>
<td>r) GEMANTH ASTHODER (Selman et Wichterman, 1937) Albaret, 1973</td>
<td>0 0 1 0 0 0 1 0 0 0 0 0 1 0 0 0 1 1 0 5</td>
</tr>
<tr>
<td>s) ALBERTIA MAXINA Affa’s, 1986</td>
<td>1 0 0 1 0 0 1 0 0 0 0 0 0 1 0 0 0 1 0 5</td>
</tr>
<tr>
<td>t) PSEUDOSCLLOPHORA CORISSLI N. GEN., N. SP.</td>
<td>1 0 0 1 0 0 0 0 1 1 0 0 0 1 0 1 0 1 1 0 8</td>
</tr>
<tr>
<td>u) NYCTOTHERIUM ANLIEL Affa’s, 1979</td>
<td>0 0 1 0 1 0 0 0 1 1 0 0 1 0 0 0 0 0 0 6</td>
</tr>
</tbody>
</table>

*See table 32 for identity of characters
### Table 34. Showing Character-State Matrix Used for Studying Phylogenetic Relationship of Geimania with Closely Related Genera

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Characters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20</td>
</tr>
<tr>
<td><em>Pronyctotherus bouchei</em></td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td><em>Nyctotheroides abcmoi</em></td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td><em>Parasricuophora xaviera</em></td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td><em>Geimania kyphodes</em></td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td><em>Albertia maxima</em></td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td><em>Pseudosricuophora corli</em></td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

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TABLE 35. SHOWING CHARACTER-STATE MATRIX OF FAMILY NYCTOTHERIDAE USED FOR CLIQUE ANALYSIS
"A" STANDS FOR PLEISOMORPHIC CHARACTER STATE & "B" STANDS FOR APOMORPHIC CHARACTER STATE

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pronyctotherus bouchei</td>
<td>1 2 3 4 5 6 7 8 11 15</td>
</tr>
<tr>
<td>Nyctotherus alpha</td>
<td>A A A A A B A A A</td>
</tr>
<tr>
<td>Pygmotheroides njinei</td>
<td>B B A A A A A A</td>
</tr>
<tr>
<td>Nyctotheroides anomalous</td>
<td>B A B A B A B A</td>
</tr>
<tr>
<td>Nyctotheroides hylae</td>
<td>B A B A B A A B</td>
</tr>
<tr>
<td>Nyctotheroides judesi</td>
<td>A A B A B A B A</td>
</tr>
<tr>
<td>Nyctotheroides abomoi</td>
<td>A A B A B A B A</td>
</tr>
<tr>
<td>Metanychtherus congol</td>
<td>B B A A A B A A</td>
</tr>
<tr>
<td>Neonyctotherus dragescoi</td>
<td>B A A B A A A A A</td>
</tr>
<tr>
<td>Taxa</td>
<td>Characters</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------</td>
</tr>
<tr>
<td><strong>Pronyctotherus bouchei</strong></td>
<td>0 0 0 0 0 0 1 0 0 0</td>
</tr>
<tr>
<td><strong>Nyctotherus alpha</strong></td>
<td>1 1 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td><strong>Pysamotheroides njinei</strong></td>
<td>1 0 1 0 0 0 1 1 1 1</td>
</tr>
<tr>
<td><strong>Nyctotheroides anomalous</strong></td>
<td>1 0 1 0 1 0 0 1 0 0</td>
</tr>
<tr>
<td><strong>Nyctotheroides hylae</strong></td>
<td>0 0 1 0 1 0 0 0 1 0</td>
</tr>
<tr>
<td><strong>Nyctotheroides judei</strong></td>
<td>0 0 1 0 1 0 0 0 0 0</td>
</tr>
<tr>
<td><strong>Nyctotheroides abomoii</strong></td>
<td>0 0 1 0 1 0 1 0 1 0</td>
</tr>
<tr>
<td><strong>Metanycotherus congoi</strong></td>
<td>1 1 0 0 0 1 0 0 0 0</td>
</tr>
<tr>
<td><strong>Neonyctotherus dragescoi</strong></td>
<td>1 0 0 1 0 0 0 0 0 0</td>
</tr>
</tbody>
</table>
1928, is represented by four species (1) *N. hylae* (Surowiak, 1937) Albaret, 1975, (2) *N. anomalous* Affa'a, 1979, (3) *N. judesi* Affa'a, 1980 and (4) *N. abomoi* Affa'a, 1980, corresponding to the four groups, namely, *Nyctotheroides* group typical, *N. group anomalus*, *N. group judesi* and *N. group abomoi* recognized recently by Affa'a (1980). The genera *Pseudonyctotherus* Earl, 1970; *Wichtermania* Earl, 1972; and *Kudoella* Earl, 1972 are not included in the present analyses because these genera are not considered valid (Albaret, 1975 & Corliss, 1979).

A data matrix was generated using twenty binary state characters (Table 33). Besides main data matrix small subsets of data matrices were also generated (Tables 34 - 35). The characters of all the taxa were polarised ('0' for plesiomorphic and '1' for apomorphic character states). This was done by out group comparison according to the method of Watrous & Wheeler (1981). The outgroups used in the present study were the members of family Metopidae Kahl, 1927 especially genus *Metopus* Claparèdè & Lachmann, 1858, because metopids are considered the most likely group from which the endocommensal heterotrichids originated (Jankowski, 1968; Albaret, 1975 and Corliss, 1979). According to Brooks et al., (1984)...

"the choice of an out-group should at least satisfy two main criteria. First the taxa of the out-group should be close enough in relation to the study taxa to allow a comparison of characters. Second, the taxa of the out-group
should be a **MONOPHyletic Linkage** outside of the study taxa' emphasis original.

The terminology followed here is that of Wiley (1981), Brooks *et al.*, (1984) and Funk (1985). Two types of character states are recognized for phylogenetic analysis. These are plesiomorphic -- which is general or more primitive state of a character in contrast to apomorphic state/s of a character which is/are considered uniquely derived (Platnic, 1979) evolutionary novelty/ies (Wiley, 1981). An apomorphic character present only in one taxon is called autoapomorphic and when shared by two or more taxa is termed synapomorphic. Synapomorphies which are considered to have been inherited from a most recent common ancestor are the basis of recognizing monophyletic groups (Henning, 1966; Wiley, 1979 & 1981 and Brooks *et al.*, 1984).

Character polarisation involves the determination of apomorphic or plesiomorphic states of a character. Many authors have discussed methods for determining plesiomorphic (= primitive) and apomorphic (=derived) character states (Maslin, 1952; Henning, 1965 & 1966; Kluge & Farris, 1969; Marx & Rabb, 1970, 1972; Lundberg, 1972; Ross, 1974; Munroe, 1974; Ekis, 1977; Eldredge, 1979; Crisci & Stuessy, 1980; Watrous & Wheeler, 1981 & Maddison *et al.*, 1984).

The following methods were used in the present
study for making phylogenetic and taxonomic inferences:

1. Farris's (1970) Wagner tree analysis
2. Character compatibility or clique analysis as recommended by Meacham (1980 & 1981).

Besides these, two optimality measures -- consistency index (Kluge & Farris, 1969) and D-measure (Brooks et al., 1986) were used to compare the relative merits of Albaret's (1975) phylogenetic tree and the trees produced by various methods in the present investigation. These methods are discussed below:

1. **FARRIS'S (1970) WAGNER TREE ANALYSIS**

Wagner tree analysis is a quantitative technique employed in the evolutionary taxonomy. The Wagner trees were constructed according to the algorithm presented by Farris (1970). The Wagner tree construction was preceded by the calculation of OTU x OTU Manhattan distance matrix. Manhattan distance between each pair of OTUs was calculated by the formula:

\[ d(A, B) = \sum_{i=1}^{n} | A_i - B_i | \]
where \( d \) = distance between two operational taxonomic units A & B and \( i \) = a given character.

The distance between an interval and an OTU not yet on a tree was calculated by the formula:

\[
d(X, \text{INT (A,B)}) = \frac{1}{2} \left\{ d(X,A) + d(X,B) - d(A,B) \right\}
\]

where \( d \) = distance between 'X' (OTU not yet placed on the tree) and the interval bound by A & B.

Finally, the advancement index \( (AD) \) for each OTU was calculated. Advancement index is the Manhattan distance of an OTU from its probable ancestor.

2. CHARACTER COMPATIBILITY OR CLIQUE ANALYSIS

Character compatibility or clique analysis is another quantitative approach for inferring phylogenies. This technique reveals patterns of agreement and disagreement between characters in a given data set (Macnam, 1981). In this technique an "appropriate sub-collection" of characters are selected which are non-conflicting as regards to the evolutionary trend. Wilson (1965) and Le Quesne (1969 & 1972) were first to suggest the use of non-conflicting character sets for inferring phylogenies. Subsequently, this technique was provided mathematical foundation by Estabrook (1972), Estabrook, Johnson & McMorris (1975, 1976 a & b), Estabrook & Landrum (1975),
Estabrook & McMorris (1977 and 1983), McMorris (1977) and Estabrook & Meacham (1979). The main disadvantage with this technique is that it is computationally difficult (Day & Sankoff, 1986). However, according to Meacham (1981) for reasonably small data sets the analysis can be done without much difficulty and "requires only time and patience".

In the present investigation character compatibility analysis was performed according to the method of Meacham (1981). The cladistic (rooted) binary data was used for performing the analysis (Table 33). All the pairs of characters were tested for compatibility. The number of comparisons made for testing compatibilities is equal to $n(n-1)/2$ where 'n' is the number of characters used. Two characters are incompatible if all the four combinations of character states are present in the data set (Meacham, 1981). After the compatibility tests the largest cliques (a set of characters that are mutually compatible) were identified. And finally after the largest cliques were found evolutionary trees were "popped up" corresponding to each clique.

Secondary character compatibility analysis was also conducted when needed. In this analysis a restricted set of taxa which were not resolved in the primary analysis were processed through the analysis again. According to Estabrook et al., (1977), "Typically primary characters suggest major lines of descent but leave unresolved
questions of relationships with them. When more resolution of detail is deserved, the \textit{primary} characters can often be augmented by secondary characters which make new distinctions. This is done by choosing a subcollection of EU's (evolutionary units) that is convex on the estimate provided by the primary characters and that contain the unresolved area.

3. CLUSTER ANALYSIS

Legendre & Legendre (1983) have defined cluster analysis as, "\ldots a\noperation of multidimensional analysis which consists of partitioning the collection of objects (or descriptors) in the study. The classification of objects (or descriptors) which result from clustering may be composed of a single partition or many hierarchial partitions of the objects (or descriptors) depending upon the clustering model used". The use of these mathematical models in taxonomy were formally introduced by Sokal & Sneath (1963) and Sneath & Sokal (1973). In the present work two types of hierarchial agglomerative clustering models were used. These are single linkage clustering and unweighted arithmetic average clustering (also known as "unweighted pair-group method with simple arithmetic averages" or "group-average sorting").

Two types of association matrices were used for clustering algorithms. These are:
a. Simple matching binary coefficient ($S_{SM}$)

$$S_{SM} (X_1, X_2) = \frac{a + d}{n}$$

where 'a' = number of characters of which the two taxa $X_1$ and $X_2$ are scored '1' and 'd' = the number of characters for which the two taxa are scored '0' and 'n' = number of total characters.

This association coefficient was introduced by Sokal & Michener (1958). This method assumes no difference between apomorphic and plesiomorphic character states and, therefore, whichever clustering method is used the relationship expressed will be phenetic (i.e., overall similarity) and may not reflect phylogeny.


$$S_J (X_1, X_2) = \frac{a}{a + b + c}$$

where 'a' = number of characters for which the two taxa $X_1$ & $X_2$ are scored '1'; 'b' and 'c' = the number of characters for which the two taxa differ (i.e. they are scored '0','1' or '1','0').

In the present work the data used were cladistic (= rooted) i.e., the characters were coded in such a way that plesiomorphic character states were scored '0' and apomorphic states were scored '1'. Since Jaccard's (1908) coefficient does not process double-zeros
i.e., does not consider plesiomorphic character states and therefore, the resulting dendrograms especially those which are generated by "UPGMA" algorithms are considered phylogenetic. Brinkhurst & Nemec (1987) have also expressed similar opinion. According to these authors, "Jaccard compares only positive matches and may be held to be Henningian in this regard if the characters are carefully coded so that "presence" is equal to apomorphic".

The above association matrices were used as starting point for both single linkage and "UPGMA" clustering models and also MST-analysis.

a. Single Linkage Clustering

According to Estabrook (1966), in single linkage clustering, a link is defined for any pairs of objects (= taxa) 'g' and 'm' by the relation $G_c$

$$\ldots$$

$G_{cm}$ if and only if $S(j,m) \geq c$

where index 'c' in the clustering relation and $G_c$ is the level of similarity considered.

The taxa are clumped hierarchically beginning with the two most mutually similar taxa followed by letting other taxa to clump into clusters and clusters with each other gradually as the similarity criterion is decreased.
b. *UPGMA* -- Unweighted *Un*-*Group Method* with simple arithmetic *Averages*.

This method was computed according to the algorithm described by Rohlf (1970) and Legendre & Legendre (1983). In *UPGMA* method the initial step is same as that of single linkage clustering. However, during the subsequent clustering cycles, the association coefficients are recalculated on the initial matrix by computing the arithmetic averages of the similarities between the taxon/taxa which is/are to be included in the new clusters.

4. **MINIMUM SPANNING TREE ANALYSIS** (MST)

Minimum spanning tree (Gower & Ross, 1969) was also calculated from Jaccard's similarity matrix (Table 33). Minimum spanning tree (MST) is similar to single linkage clustering except that the taxa are connected by primary links only. According to Rohlf (1982), for a set of *n* p-dimensional vertices (objects), MST consists of *n*-1 edges (connections), joining the pairs of objects in such a way that no closed loops occur and each object is at least connected to another object by an edge (connection) and the length of the tree is as minimum as possible.

5. **FARRIS'S (1970) TREE OPTIMIZATION PROCEDURE**

Farris's tree optimization procedure was employed to increase the parsimony of the trees generated by
Wagner analysis and minimum spanning tree analysis. The dendrograms constructed by 'UPGMA' clustering technique were also subjected to hypothetical taxonomic unit (HTU) optimization as recommended by Farris (1970). According to the principle of parsimony, the shortest length trees are given preference, that is, those trees which make "fewest assumptions of reversals and parallelism as possible" (Funk, 1985). The principle of parsimony does not say that evolution is parsimonious but demands "economy of assumptions in reasoning" (Brooks et al., 1984).

6. OPTIMALITY MEASURES

a) Consistency Index (CI)

Consistency index (Kluge & Farris, 1969) was used to find the amount of fit of the trees to the data. The CI was measured as the ratio of minimum number of steps required to explain the data ÷ the actual tree length. Its value lies between 0 - 1 with 1 indicating a perfect fit of the tree to the data.

b) The D-Measure

The D-measure was introduced by Brooks et al., (1986). According to these authors the D-measure, "... quantifies the amount of historical constraint on character evolution postulated by phylogenetic hypothesis". The D-measure was used to estimate the information content of the phylogenetic
trees and also as an optimality criterion for selecting between two or more equally parsimonious minimum length trees. The D-measure of the trees was calculated as the difference between the total information capacity of the data \( H_{\text{max}} \) and the actual symbol information content \( H_S \) of the characters present on the trees.

\[ H_S = \sum_{i=1}^{n} P_i \log_2 P_i \]

where \( H_S \) is the information content of the symbols (character states) in an array and \( P_i \) is fraction where numerator of \( P_i \) is the number of times particular symbol appears in the array and denominator is the total number of symbols in an array \( A \). The total information capacity of the array was calculated by the formula:

\[ H_{\text{max}} = -A \left( \sum_{A} \frac{i}{A} \log_2 \frac{i}{A} \right) = \log_2 A \]

The D-measure was calculated as:

\[ D = H_{\text{max}} - H_S \]

or

\[ D = \log_2 A - H_S \]

The value of \( D \) ranges from '0' to any positive value depending upon the size of the data sets. Therefore, Brooks et al., (1986), proposed to use R-value, suggested earlier by Gatlin (1972), in order to get a
normalized measure.

\[
R = \frac{D}{\log_2 A}
\]

The value of \( R \) ranges from 0 to 1.

**RESULTS AND DISCUSSION**

The results of the single linkage and "UPGMA" clustering and minimum spanning tree analysis (MST) of the entire data set (i.e. twenty-one terminal taxa) are given in Figs. 68-70. It is interesting to note that all the three methods followed in the present investigation do not support the natural existence of family Sicuophoridae (Amaro, 1972) Albaret, 1975 as distinct from family Nyctotheridae Amaro, 1972. This is because of two reasons given as under:

1. The genus *Nyctosita* Amfa'a, 1979 inspite of having a sucker armed with polysaccharide skeletal elements always clusters with (on the basis of other synapomorphies) the members of genera *Nyctotheriodes* Grassé, 1928 and *Pymotheroides* Affa'a, 1979 and does not cluster with sicuophorids. According to Albaret (1975) and Corliss (1973), the only difference between families Sicuophoridae and Nyctotheridae is that the sucker is armed with polysaccharide skeletal elements in the members of family Sicuophoridae.

2. The genera *Nyctotherus* Leidy, 1849 and *Metanyctotherus* Albaret, 1970, cluster with the members of
family Sicuophoridae even though they are devoid of an armed sucker as shown in all the three analyses.

Grouping the genera of heterotrichid ciliates into two distinct families, Nyctotheridae and Sicuophoridae, is not justifiable as it would accentuate deceptive affinities. Therefore, in the present investigation the family Sicuophoridae (Amaro, 1972) Albaret, 1975 is suppressed and is considered a junior synonym of family Nyctotheridae.

In single linkage and 'UPGMA' clustering models, Sicuophora heimi Albaret, 1970 and S. macropharyngea (Bezzenberger, 1903) Albaret, 1975 always cluster together. However, S. magna (Bezzenberger, 1904) Albaret, 1975 (= S. mabokensis Albaret, 1970) does not cluster with S. heimi and S. macropharyngea and instead is more closely associated with Neosicuophora n.gen. This means that the genus Sicuophora de Puytorac & Grain, 1968, sensu Albaret (1975), is not a homogenous assemblage of species and, therefore, S. magna should be given a separate generic status. A close relationship between Nyctotheroides abomai Affa'a, 1980; N. hylae (Surowiak, 1937) Albaret, 1975; N. judesi Affa'a, 1980; and N. anomalus Affa'a, 1979 was originally suggested by Affa'a (1980). Both the single linkage clustering as well as 'UPGMA' method support this view. The present study also suggests a close relationship between Metanyctotherus and Nyctotherus.
as already suggested by Albaret (1975). Genus Geimania Albaret, 1975 did not seem to have a fixed place. In 'UPGMA', Geimania clustered with Pronyctotherus Albaret & Njine, 1975 whereas, in single linkage clustering, Geimania clustered with the group formed by Sicuophora de Puytorac & Oktem, 1957; Parasicuophora Albaret, 1958; Sicuophoroides n.gen.; Neosicuophora n.gen.; Pseudosicuophora n.gen.; Prosicuophora de Puytorac & Oktem, 1957; Metasicuophora Albaret, 1973 and Albertia Affa'a, 1986 whereas, in MST, Geimania is closely linked to Albertia. To solve this discrepancy, two cladistic methodologies, namely, Wagner tree analysis and clique analysis were performed. Six taxa were included in these analyses which were found close to Geimania. In clique analysis, two largest equally competing cliques, consisting of eight characters, were obtained (Fig. 71A, B). Incidentally, in Wagner tree analysis also, two equally parsimonious trees were generated (Figs. 72A & B). Both the Wagner trees (Figs. 72A, B) and one of the clique analysis (Fig. 71A) do not support the close relationship of Geimania with Albertia and instead support a close relationship of Geimania with Pronyctotherus.

Two previously unrecognised relationships between taxa Neonyctotherus Affa'a, 1983 with Sicuophora magna and Metasicuophora with Prosicuophora are also suggested by the present study (UPGMA; single linkage clustering techniques; and MST analyses).
Nine taxa belonging to the family Nyctotheridae sensu Albaret, 1975 were subjected to two conceptually different phylogenetic methods, namely, clique analysis (Fig. 73A-D) and 'UPGMA' (Fig. 74) based upon synapomorphies. Both these methods separated Metanyctotherus, Neonyctotherus, and Nyctotherus from other members of this family corroborating the already expressed view by the present author which was based on the entire data set.

In the present investigation the single linkage clustering algorithm which has space contraction property (Legendre & Legendre, 1983) was used because of its Hennigian acceptability (Brinkhurst & Nemec, 1987). However, single linkage clustering has a disadvantage of chaining effect. Therefore, another conceptually different algorithm 'UPGMA', which is space indifferent was used to supplement the results generated by single linkage clustering. Smith & Phipps (1984) recommended the use of consensus tree analysis for evaluating complex group structures. Therefore, in the present investigation Neumann's (1983) 'durchschnitt consensus method' was used to evaluate the amount of information shared by the two phylogenetic hypotheses generated by the two conceptually different algorithms. The results of this analysis is summarized in Fig. 76. The consensus tree does not also support the existence of the family Sicuophoridae as distinct from family Nyctotheridae. Besides, the above consensus tree also recognizes Sicuophora magna (Hezzenbergeri.
Figure 70. Full dimensional minimum spanning tree (MST) rooted at *Pronyctotherus* showing relationship among endocommensal heterotrichid ciliates.
Figures 71 A & B. Showing phylogenetic relationships of Geimania with other related genera. These phylogenetic trees are based on two equally competing cliques resolved by character compatibility analysis. For identity of characters and taxa refer to tables 32 and 33, respectively. Numbers indicate characters and letters indicate taxa.
Figures 72 A & B. Two equally parsimonious Wagner trees showing phylogenetic relationship of *Geimania* with other related genera.
1904) Albaret, 1975 as a separate taxon and does not support its association with the other members of the genus *Sicuophora* de Puytorac & Grain, 1968, namely, *S. heimi* Albaret, 1970 and *S. macropharyngea* (Bezzenberger, 1903) Albaret, 1973. In *S. magna*, the distal tip of the infundibulum coils upon itself and makes at least one complete turn. Moreover, ventral and lateral kineties do not participate in the formation of caudal suture on the face inferior, which is not the case with the other members of the genus *Sicuophora*. Thus it appears that *S. magna* should be placed in a new genus and the author proposes the name *Bezzenbergeria* n.gen. for it. Therefore, the new name of *S. magna* is *B. magna* (Bezzenberger, 1904) n.comb.

Consensus trees were first introduced by Adams (1972) to summarize the information from two or more different classifications of the same set of taxa. Neumann's (1983) 'durchschnitt consensus method' belongs to the category of cluster intersection methods working on the principle of set theory. This method was chosen because it has a property to return more number of consensus clusters in contrast to strict consensus (Sokal & Rohlf, 1981) and Adams-II consensus method (Adams, 1972) and its use has been strongly recommended by Smith & Phipps (1984).

Farris (1973) noted that MST based on OTU x OTU distances is a close approximation to phylogenetic
hypothesis. The present results also support this view (Fig. 70).

The hypothetical taxonomic units (HTU) optimization procedure was used to find the length and to display all the character state changes on the various phylogenetic trees given in the present investigation. Of the three trees constructed for the entire data set (Figs. 68-70), only 'UPGMA' and 'MST' based trees (Figs. 77-78) were found to be of minimum length consisting of 43 steps each (i.e. two equally parsimonious trees were found). Both trees have a consistency index of 46%. In order to choose among these two trees, 'D' measure was used as an optimality criterion. The maximum 'D' value 4.50 was found in 'UPGMA' based phylogenetic tree (Fig. 77) in contrast to 'MST' (D = 4.00; Fig. 78). However, 'MST' can also be considered as a close approximation of phylogenetic hypothesis as suggested by Farris (1979).

In the present work, an attempt was made to graphically depict spatial relationship in endocommensal heterotrichid ciliates. The 3-dimensional graph, produced (Fig. 79), shows relationship of each taxon with its nearest relative (based upon twenty dimensional Jaccard's coefficient association matrix of synapomorphies) and also with Pronyctotherus from which the whole group possibly evolved. The height of the lines indicate advancement index (i.e. the number of apomorphies). A full dimensional 'MST' has been superimposed upon this graph to complement
the effect because when twenty dimensional relationship is shown in three dimensions it is bound to produce some distortion due to space contraction effect. The results of such a display are interesting. It is possible to examine this relationship with regards to both phenetic and cladistic relationship. For example, Metanvctotherus - Nyctotherus and Nyctotheroides - Pygmootheroides evolved as two divergent lines as shown in the graph by 'MST' links. However, the "horse-shoe" shaped nature of this representation shows that these taxa have undergone convergent evolution and this would also explain why some taxonomists (e.g., Albaret, 1975) grouped Metanvctotherus and Nyctotherus along with Nyctotheroides group.

Finally, the results obtained in the present investigation were compared with the results of Albaret's (1975) phylogenetic tree. Since the 'UPGMA' based tree was more informative (cf. the results of 'D' measure), therefore, this phylogenetic tree will be used for comparison with Albaret's (1975) phylogenetic tree. However, only those taxa were selected which were known to Albaret (1975) at that time to facilitate comparison (Fig. 80). Fig. 80 represent the phylogeny given by Albaret (1975). This figure was redrawn in the form of a cladogram in order to facilitate the display of all the character state changes on the tree (Fig. 81). For this Albaret's (1975) phylogenetic tree was assigned "parsimony-optimal" set of hypothetical units as recommended.
Figures 73 A, B, C & D. Phylogenetic relationships among the members of family Nyctotheridae sensu Albaret, 1975. These phylogenetic trees are based on four equally competing cliques resolved by character compatibility analysis. For identity of characters and taxa refer to tables 32 and 33, respectively. Numbers indicate characters and letters indicate taxa.
Fig. 73 b. Evolutionary tree of family Nyctotheridae sensu Albaret, 1975 generated by secondary character compatibility or clique analysis of figure 73 a. The numbers on the internodes indicate characters. For identity of characters refer to table 32.
Figure 74. Representing phylogenetic relationship and subclusters of the family Nyctotheridae sensu Albaret, 1975 (constructed according to "UPGMA" clustering technique using Jaccard's similarity matrix as a starting point).
FIG. 74

METANYCTOTHERUS CONGOI
NYCTOTHERUS ALPHA
NEONYCTOTHERUS DRAGESCOI
NYCTOTHEROIDES ABOMOI
NYCTOTHEROIDES HYLAE
NYCTOTHEROIDES JULASI
NYCTOTHEROIDES ANOMALOUS
PYGMOITHEROIDES NJINEI
PRONYCTOTHERUS BOUCHEI

Time scale in millions of years ago (Ma): 0.00 - 80
Figure 75. A phenogram (= dendrogram) representing phenetic relationship and subclusters of the family Nyctotheridae sensu Albaret, 1975 (constructed according to "UPGMA" clustering technique using simple similarity association matrix as a starting point).
Figure 75. Consensus tree (constructed according to Neumann's (1983) durchschnitt (= intersection) method) of entire data set (= 21 taxa) showing shared information between "JPGmA" and single linkage clustering based phylogenetic trees (see Figs. 68 & 69).
Figure 77. Showing phylogenetic relationship (constructed according to "JPoMIA" algorithm based on Jaccard's matrix) among heterotrichid ciliates belonging to families Sicuophoridae and Nyctotheridae. Numbers at branches represent character state changes resolved by HTU - optimization. Single lines indicate synapomorphies, double lines indicate parallelisms, 'X' indicates reversal of a synapomorphy. For identity of characters refer to table 32.
Figure 78. Showing phylogenetic relationship (based on minimum spanning tree using Jaccard's association matrix) among heterotrichid ciliates belonging to the families Sicuophoridae and Nyctotheridae. Numbers and branches represent character-state changes resolved by HTU-optimization. Single lines indicate synapomorphies, double lines indicate parallelisms and 'X' indicates reversals of synapomorphies. For identity of characters refer to table 32.
Figure 79. Spatial representation of phenetic and evolutionary relationship in endocommensal ciliate genera. This three-dimensional graph shows relationship of each taxon with its nearest relative and also with taxon 'a' (Pronyctotherus) from which the whole group possibly evolved. The height of the line indicates advancement index (AI) (i.e. number of apomorphies). A full dimensional minimum spanning tree has been superimposed.
Fig. 79
Figure 80. Albaret's (1975) original phylogenetic tree for families - Nyctotheridae & Sicuophoridae.
Figure 81. Phylogenetic relationship of heterotrichid ciliates derived from Albaret (1975). The character state changes were resolved in the present investigation by Farris's (1970) HTU - optimization procedure.
Figure 82. Phylogenetic relationship of the above taxa (Fig. 81) derived from present analysis.

Note: Albaret's (1975) Phylogenetic tree (Fig. 81) has a length of 28 steps which indicates relatively high level of parallel and convergent evolution in contrast to the present tree (Fig. 82) which has a length of only 22 steps and has a consistency index of 0.72 indicating relatively low level of parallel and convergent evolution.
Sicuophora macropharyngea
Parasicuophora xaviera e
Prosicuophora basoglu i
Metasicuophora petteri
Nyctotherus alpha
Metanyctotherus connoi
Nyctotheroides hylae
Geimania kyphodes
Pronyctotherus bouchei
by Farris (1970). But the redrawn cladogram represents accurately the topology intended by its original author. Albaret's (1975) phylogenetic tree (Fig. 81) has a length of 28 steps with consistency index of 0.57 which indicates relatively high level of parallel and convergent evolution in contrast to the present tree which has a length of only 22 steps and has a consistency index of 0.72 indicating relatively low level of parallel and convergent evolution. Thus, Albaret's tree does not describe the evolution of various morphological characters as accurately (i.e. parsimoniously) as is shown by the present tree (Fig. 82).

A key to all the known genera of endocommensal heterotrichid ciliates, is given elsewhere (pp. 231). This key is based upon the phylogram shown in Fig. 70.

CONCLUSIONS

1. The phylogenetic analysis presented in Fig. 77 is a summary of most of the knowledge known about endocommensal heterotrichid ciliates at the present time and also presents the most likely hypothesis of genealogical relationship of these organisms (constructed on the basis of synapomorphies).

2. The results of the present study (based on reasonably wide range of methodologies) show that:

a. the existence of family Sicuophoridae (Amaro,
1972) Albaret, 1975 is artificial and, hence clearly untenable. Therefore, in the present investigation the family *Sicuophoridae* is suppressed, and

b. *Sicuophora magna* (Bezzenberger, 1904) Albaret, 1975 is comparatively more closely related to the genus *Neonyctotherus* Affa'a, 1983 than it is with other members of the genus *Sicuophora*. Therefore, a new generic name, namely, *Bezzenbergeria* n.gen. is created for its reception.