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## A REVISED CLASSIFICATION OF THE PHYLUM CILIOPHORA DOFLEIN, 1901\*

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Revisión, de la Clasificación del Phylum Ciliophora Doflein, 1901

DENIS H. LYNN\*\*, EUGENE B. SMALL\*\*\*

\* We are pleased to dedicate this paper to our friend and colleague Dr: Eucario López-Ochoterena in celebration of his 30th anniversary as a Ph.D. in Biology, in recognition of his substantial contributions to protozoological research and education, and in honour of his 10 years as editor of the Revista.

\*\* Department of Zoology, University of Guelph, Guelph, ON, CANADA N1G, 2W1

\*\* Department of Zoology, University of Maryland, College Park, MD, U.S.A. 20742

### ABSTRACT

The major refinements of a revised classification of the phylum Ciliophora are reviewed, including a comparison to the classifications of Small & Lynn (1981, 1985) and de Puytorac *et al.* (1993). The newer higher taxa are justified by new observations and reference to the recent literature. Major changes include a change in the subphyla with recognition of only two: the subphylum Postciliodesmatophora Gerassimova & Seravin, 1976 and the subphylum Intramaeronucleata Lynn, 1996. The plagiopylid ciliates are elevated from subclass to class Plagiopylea. Unlike de Puytorac *et al.*, no superclasses are recognized. Ten classes of ciliates are presented and briefly discussed.

Keyword: Phylum Ciliophora, classification, Doflein, 1901

### RESUMEN

Los mayores logros de una revisión de la clasificación del Phylum Ciliophora se hicieron al comparar las clasificaciones de Small & Lynn (1981, 1985) y de Puytorac *et al.* (1993). Los nuevos taxa superiores son justificados por observaciones y referidos a la literatura reciente. El mayor cambio elimina uno de los subphyla por lo que solo se reconocen dos: el subphylum Postciliodesmatophora Gerassimova & Seravin, 1976 y el subphylum Intramacronucleata Lynn, 1996. Los ciliados plagiopilidos son elevados de subclase a clase Plagiopylea. A diferencia de Puytorac *et al.* (1993), no se reconocen las superclases. Diez clases de ciliados son presentadas y brevemente discutidas.

Palabras clave: Phylum ciliophora, clasificación, Doflein, 1901

### Introduction

Small and Lynn (1981) presented a new macrosystem for the Phylum Ciliophora Doflein, 1901 based principally on the structural conservatism of the kinetidal components in the somatic cortex. The stability of somatic kinetic patterns, reviewed by Lynn (1981) and more recently by Lynn (1991), was used to recognize major clades within the phylum. Small and Lynn (1981) also considered that division morphogenesis was a significant feature, especially for

recognizing diversity within these major clades. They suggested that there appeared to be more stability in the dikinetid structures of the oral region, than the polykinetid structures, based on an analysis of Small (1976) who recognized two basic oral dikinetids: ones whose transverse microtubular ribbons were cytostomially directed and the other whose postciliary microtubular ribbons were cytostomially directed. Finally, Small and Lynn (1981) favoured Raikov's hypothesis (Raikov, 1969) explaining the evolution of nuclear dualism in the phylum.

With greater weight being placed on the conservation of somatic kinetic structures and oral dikinetids, Small and Lynn (1981, 1985) proposed three subphyla and eight classes within the phylum (Table 1). Three of the classes, the LITOSTOMATEA, NASSOPHOREA, and COLPODEA, were new and 10 new orders were also defined. Since 1981, the French ciliatologists have published two revisions to their 1974 classification of the ciliates (de Puytorac *et al.*, 1974) - de Puytorac *et al.* (1987) and de Puytorac *et al.* (1993). The latter classification is presented in a slightly revised form by de Puytorac (1994) and will be discussed below (Table 2). Our analysis of the evidence, which includes descriptions of the ultrastructure of both morphostatic and morphogenetic states and analyses of gene sequences, particularly the small (SSrRNA) and large subunit ribosomal RNA (LSrRNA) genes, leads us to conclusions different from our French colleagues. We present and explain below our revised higher classification of the phylum Ciliophora (Table 3). We limit our discussion here to taxa at the ordinal level and above.

Table 1. Classification of the Phylum Ciliophora proposed by Small & Lynn (1985).

Phylum CILIOPHORA Doflein, 1901

Subphylum *POSTCILIODESMATOPHORA* Gerassimova & Seravin, 1976

Class KARYORELICTEA Corliss, 1974

- Order Protostomatida Small & Lynn, 1985
- Order Loxodida Jankowski in Small & Lynn, 1985
- Order Protoheterotrichida Nonzarède, 1977
- Order Protocruziida Jankowski in Small & Lynn, 1985\*

Class SPIROTRICHEA Bütschli, 1889

- Subclass Heterotrichia Steni, 1859B
  - Order Heterotrichida Stein, 1859
  - Order Clevelandellida de Puytorac & Grain, 1976
  - Order Armophorida Jankowski, 1964
  - Order Plagiotomida Albaret, 1974
  - Order Phacodiniida Small & Lynn, 1985
  - Order Odontostomatida Sawaya, 1940
  - Order Licnophorida Corliss, 1957

*Incertae sedis*

- Order Peritromida Jankowski in Small & Lynn, 1985B
- Subclass Stichotrichia Small & Lynn, 1985
  - Order Stichotrichida Fauré-Fremiet, 1961
- Subclass Choreotrichia Small & Lynn, 1985
  - Order Choreotrichida Small & Lynn, 1985\*
  - Order Oligotrichida Bütschli, 1887\*

Subphylum *RHABDOPHORA* Small, 1976

Class PROSTOMATEA Schewiakoff 1896

- Order Prostomatida Schewiakoff 1896
- Order Prorodontida Corliss, 1974

Class LITOSTOMATEA Small & Lynn, 1981

- Subclass Haptoria Corliss, 1974
  - Order Haptorida Corliss, 1974
  - Order Pleurostomatida Schewiakoff, 1896
  - Order Pharyngophorida Small & Lynn, 1985B
- Subclass Trichostomatia Bütschli, 1889
  - Order Vestibuliferida de Puytorac *et al.*, 1974
  - Order Entodiniomorphida Reichenow in Doflein & Reichenow, 1929

- Subphylum *CYRTOPHORA* Small, 1976
- Class PHYLLOPHARYNGEA de Puytorac *et al.*, 1974
    - Subclass Phyllopharyngia de Puytorac *et al.*, 1974
      - Order Cyrtophorida Fauré-Fremiet in Corliss, 1956B
      - Order Rhynchodida Chatton & Lwoff, 1939\*
    - Subclass Chonotrichia Wallengren, 1895
      - Order Exogemmida Jankowski, 1972
      - Order Cryptogemmida Jankowski, 1975
    - Subclass Suctoria Claparède & Lachmann, 1858
      - Order Exogenida Collin, 1912
      - Order Endogenida Collin, 1912
      - Order Evaginogenida Jankowski in Corliss, 1979
  - Class NASSOPHOREA Small & Lynn, 1981
    - Subclass Nassophoria Small & Lynn, 1981B
      - Order Synhymeniida de Puytorac *et al.*, 1974
      - Order Nassulida Jankowski, 1967
      - Order Microthoracida Jankowski, 1967
      - Order Propeniculida Small & Lynn, 1985B
      - Order Peniculida Fauré-Fremiet in Corliss, 1956\*
    - Subclass Hypotrichia Stein, 1859
      - Order Euplotida Small & Lynn, 1985
  - Class COLPODEA Small & Lynn, 1981
    - Order Cyrtolophosidida Foissner, 1978
    - Order Bryophryida de Puytorac *et al.*, 1979
    - Order Colpodida de Puytorac *et al.*, 1974
    - Order Bursariomorphida Fernández-Galiano, 1978
  - Class OLIGOHYMENOPHOREA de Puytorac *et al.*, 1974
    - Subclass Hymenostomatia Delage & Hérouard, 1896
      - Order Hymenostomatida Delage & Hérouard, 1896
      - Order Scuticociliatida Small, 1967\*
    - Subclass Peritrichia Stein, 1859
      - Order Sessilida Kahl, 1933
      - Order Mobilida Kahl, 1933
    - Subclass Astomatia Schewiakoff, 1896
      - Order Astomatida Schewiakoff 1896
    - Subclass Apostomatia Chatton & Lwoff, 1928
      - Order Apostomatida Chatton & Lwoff, 1928
      - Order Astomatophorida Jankowski, 1966
      - Order Pilisuctorida Jankowski, 1966
    - Subclass Plagiopylia Small & Lynn, 1985B
      - Order Plagiopylida Small & Lynn, 1985

\* These taxa have been elevated to subclass rank in the revised system. See Table 3.

BThese taxa have been eliminated from the revised system and included taxa have been transferred to related groups. See text for explanation.

#### How Many Superphyla?

Small and Lynn (1981, 1985) divide the phylum into three subphyla: the *POSTCILIODESMATOPHORA* Gerassimova & Seravin, 1976, distinguished principally by the presence of postciliodesmata in the somatic cortex (Gerassimova and Seravin, 1976; Small and Lynn, 1981, 1985); the *RHABDOPHORA* Small, 1976, distinguished by the presence of transverse microtubules supporting the cytopharynx and by the *rhabdos* as the cytopharyngeal apparatus (Small, 1976); and the *CYRTOPHORA* Small, 1976, distinguished by the presence of postciliary microtubules supporting the cytopharynx and, at least in presumed ancestral forms, by the *cyrtos* as the

cytopharyngeal apparatus (Small, 1976).

Table 2. Classification of the Phylum Ciliophora proposed by de Puytorae (1994).

- Phylum CILIOPHORA Doflein, 1901
  - Subphylum *TUBULICORTICATA* de Puytorac *et al.*, 1993
    - Superclass POSTCILIODESMATOPHORA Gerassimova & Seravin, 1976
      - Class KARYORELICTEA Corliss, 1974
        - Subclass Trachelocercia Jankowski, 1980
          - Order Trachelocercida Kent, 1881
        - Subclass Loxodia Jankowski, 1980
          - Order Loxodida Jankowski in Small & Lynn, 1985
        - Subclass Protocruziidia Jankowski in Small & Lynn, 1985
          - Order Protocruziida Jankowski in Small & Lynn, 1985
        - Subclass Protoheterotrichia Nonzarède, 1977
          - Order Protoheterotrichida Nouzarède, 1977
      - Class HETEROTRICHEA Stein, 1859
        - Subclass Heterotrichia Stein, 1859
          - Order Heterotrichida Stein, 1859
          - Order Coliphorida Jankowski, 1967
          - Order Armophorida Jankowski, 1964
          - Order Phacodiniida Small & Lynn, 1985
          - Order Odontostomatida Sawaya, 1940
          - Order Licnophorida Corliss, 1957
        - Subclass Clevelandellidia de Puytorac & Grain, 1976
          - Order Clevelandellida de Puytorac & Grain, 1976
    - Superclass SPIROTRICEIA Bütschli, 1889
      - Class HYPOTRICHEA Stein, 1859
        - Subclass Euplotia Tuffrau & Fleury in de Puytorac *et al.*, 1993
          - Order Kiitrichida Nozawa, 1941
          - Order Euplotida Small & Lynn, 1985
          - Order Uronychiida Tuffrau & Fleury in de Puytorac *et al.*, 1993
          - Order Gastrocirrhida Tuffrau & Fleury in de Puytorac *et al.*, 1993
        - Subclass Oxytrichia Tuffrau & Fleury in de Puytorac *et al.*, 1993
          - Order Urostylida Jankowski, 1979
          - Order Discocephalida Wicklow, 1982
          - Order Oxytrichida Jankowski, 1979
      - Class OLIGOTRICHEA Bütschli, 1887
        - Subclass Oligotrichia Bütschli, 1887
          - Order Oligotrichida Bütschli, 1887
        - Subclass Strobilia Laval-Peuto, Grain & Deroux in de Puytorac *et al.*, 1993
          - Order Choreotrichida Small & Lynn, 1985
          - Order Tintinnida Kofoid & Campbell, 1929
    - Superclass TRANSVERSALA de Puytorac *et al.*, 1993
      - Class COLPODEA Small & Lynn, 1981
        - Subclass Colpodia Foissner, 1985
          - Order Colpodida de Puytorac *et al.*, 1974
          - Order Grossglockneriida Foissner, 1985
          - Order Bursariomorphida Fernández-Galiano, 1978
          - Order Cyrtolophosidida Foissner, 1978
          - Order Sorogenida Foissner, 1985
          - Order Bryophryida de Puytorac *et al.*, 1979
        - Subclass Bryometopia Foissner, 1985
          - Order Bryometopida Foissner, 1985

Class PLAGIOPYLEA Small & Lynn, 1985  
Order Plagiopylida Small & Lynn, 1985

Subphylum *FILICORTICATA* de Puytorac *et al.*, 1993

Class LITOSTOMATEA Small & Lynn, 1981  
Order Haptorida Corliss, 1974  
Order Spathidiida Foissner & Foissner, 1988  
Order Helicoprordontida Grain in de Puytorac *et al.*, 1993  
Order Pleurostomatida Schewiakoff, 1896  
Order Mesodiniida Grain in de Puytorac *et al.*, 1993  
Class VESTIBULIFEREA de Puytorac *et al.*, 1974  
Order Trichostomatida Butschli, 1889  
Order Entodiniomorphida Reichenow in Doflein & Reichenow, 1929  
Order Blepharocorythida Wolska, 1971

Subphylum *EPIPLASMATA* de Puytorac *et al.*, 1993

Superclass CILIOSTOMATOPHORA de Puytorac *et al.*, 1993  
Class PHYLLOPHARYNGEA de Puytorac *et al.*, 1974  
Subclass Cyrtophoria Faure-Fremiet in Corliss, 1956  
Order Chilodonellida Deroux in de Puytorac *et al.*, 1993  
Order Chlamyodontida Deroux in de Puytorac *et al.*, 1993  
Order Dysteriida Deroux in de Puytorac *et al.*, 1993  
Subclass Chonotrichia Wallengren, 1895  
Order Chilodochonida Batisse in de Puytorac *et al.*, 1993  
Order Exogemmida Jankowski, 1972  
Order Cryptogemmida Jankowski, 1975  
Subclass Rhynchodia Chatton & Lwoff, 1939  
Order Hypocomatida Deroux, 1976  
Order Rhynchodida Chatton & Lwoff 1939  
Subclass Suctoria Claparede & Lachmann, 1858  
Order Podophryida Jankowski, 1973  
Order Exotropida Batisse in de Puytorac *et al.*, 1993  
Order Entotropida Batisse in de Puytorac *et al.*, 1993

Superclass MEMBRANELLOPHORA Jankowski, 1975

Class NASSOPHOREA Small & Lynn, 1981  
Subclass Prostomatia Schewiakoff, 1896  
Order Prostomatida Schewiakoff, 1896  
Order Prorodontida Corliss, 1974  
Subclass Nassulia Jankowski, 1967  
Order Nassulopsida Deroux in de Puytorac *et al.*, 1993  
Order Synhymeniida Deroux, 1978  
Order Nassulida Jankowski, 1967  
Order Paranassulida Deroux in de Puytorac *et al.*, 1993  
Order Parahymenostomatida Grain *et al.*, 1976  
Order Microthoracida Jankowski, 1967

Class OLIGOHYMENOPHOREA de Puytorac *et al.*, 1974

Subclass Peniculia Fauré-Fremiet in Corliss, 1956  
Order Peniculida Faure-Fremiet in Corliss, 1956  
Order Urocentrida de Puytorac *et al.*, 1987  
Subclass Scuticociliatia Small, 1967  
Order Philasterida Small, 1967  
Order Pleuronematida Fauré-Fremiet in Corliss, 1956  
Subclass Peritrichia Stein, 1859  
Order Sessilida Kahl, 1933  
Order Mobilida Kahl, 1933  
Subclass Hysterocinetia Diesing, 1866  
Order Hysterocinetida Diesing, 1866

Subclass Astomatia Schewiakoff, 1896  
  Order Hoplitophryida Cheissin, 1930  
  Order Anoplophryida Cépède, 1910  
Subclass Hymenostomatia Delage & Herouard, 1896  
  Order Tetrahymenida Faure-Fremiet in Corliss, 1956  
  Order Ophryoglenida Canella, 1964  
Subclass Apostomatia Chatton & Lwoff 1928  
  Order Apostomatida Chatton & Lwoff 1928  
  Order Astomatophorida Jankowski, 1966  
  Order Pilisuctorida Jankowski, 1966

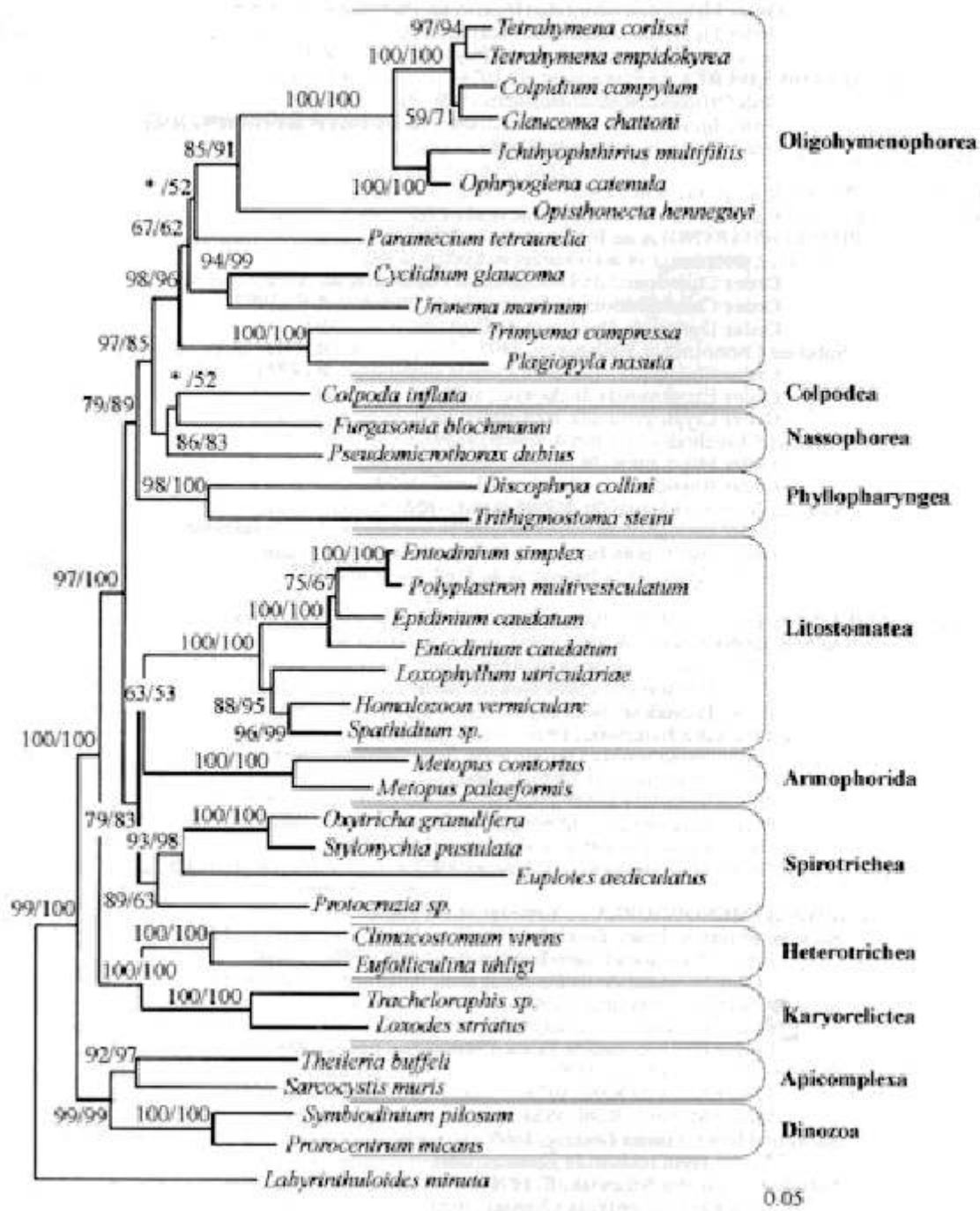


Fig.1. A distance tree for the ciliates derived from evolutionary distances between SSrRNA gene sequences produced by the Jukes-Cantor correction model and constructed using the Fitch-Margoliash least squares (LS) method and neighbour-joining (NJ) method. The LS bootstrap percent for 500 resamplings is followed by the NJ bootstrap percent at the base of each clade. Bootstrap percents less than 50% are indicated by an asterisk (\*). Evolutionary distance is represented by the horizontal component separating species in the figure. The scale bar corresponds to 5 changes per 100 positions. Names for suprageneric taxa appear in boldface. From Wright *et al.*,

1997.

In a detailed study of the ultrastructure of stomatogenesis, Huttenlauch and Bardele (1987) demonstrated that *Coleps*, a typical prostomate rhabdophoran, had postciliary microtubules supporting the cytopharynx rather than the transverse microtubules that observation of the morphostatic state had led previous researchers to believe (Lynn, 1985; de Puytorac and Grain, 1972). This removed the class PROSTOMATEA, one of the two included classes from the subphylum RHABDOPHORA, and undercut confidence in observation of morphostatic characters. Analysis of the literature also indicated that many taxa within both the subphyla POSTCILIODESMATOPHORA and CYRTOPHORA had postciliary microtubules supporting the cytopharynx (Lynn and Corliss, 1991). Thus, aside from the presence of the *cyrtos* in only some members of the subphylum CYRTOPHORA (i.e., class NASSOPHOREA), there were no strong characters separating cyrtophorans and postciliodesmatophorans. For these reasons, Lynn and Corliss (1991) abandoned the subphyla of Small and Lynn (1981, 1985).

De Puytorac *et al.* (1993) proposed three new subphyla, differing from those of Small and Lynn (1981, 1985). De Puytorac (1994) retains these three subphyla (Table 2). They based their conclusions on phylogenies derived from partial LSrRNA gene sequences (Baroin *et al.*, 1992) and on an analysis of the evolution of the cytoskeleton (Fleury *et al.*, 1992). The subphylum TUBULICORTICATA de Puytorac *et al.*, 1993 was characterized as having a cortical cytoskeleton of superficial microtubules associated, or not, with cortical kinetosomes (de Puytorac *et al.*, 1993). The subphylum FILICORTICATA de Puytorac *et al.*, 1993 was distinguished by a cortex with a microfibrillar ecto-endoplasmic boundary (i.e., the *tela corticalis*) that might or might not be continuous and that could be composed of one or two layers (de Puytorac *et al.*, 1993). This subphylum essentially corresponded to the subphylum RHABDOPHORA Small, 1976 with the class PROSTOMATEA removed. The third subphylum EPIPLASMATA de Puytorac *et al.*, 1993 included ciliates with a fibro-granular epiplasmic layer that might or might not be continuous beneath the inner alveolar membrane (de Puytorac *et al.*, 1993). Like the subphyla proposed by Small and Lynn (1981, 1985), there are problems with the characterizations of these subphyla of de Puytorac *et al.* (1993). First, virtually all ciliates could be described as having a "cortical cytoskeleton of superficial microtubules associated, or not, with cortical kinetosomes" (i.e., be members of the subphylum TUBULICORTICATA). Second, many members of the subphylum TUBULICORTICATA have a very well-developed fibrous epiplasm, and could therefore easily be members of the subphylum EPIPLASMATA (e.g., some colpodeans, see Lynn, 1976a, 1978). Finally, the parsimony analysis of de Puytorac *et al.* (1994) demonstrated that both the TUBULICORTICATA and EPIPLASMATA could be paraphyletic groups (Figs. 1, 2 in de Puytorac *et al.*, 1994).

There is ample evidence now from a variety of independent phylogenetic analyses of both SSrRNA and LSrRNA genes that there is a fundamental bifurcation (Fig. 1) in the ciliates (e.g., Baroin-Tourancheau *et al.*, 1995; Hammerschmidt *et al.*, 1996; Hirt *et al.*, 1995; Wright *et al.*, 1997). This bifurcation, having close to 100% bootstrap support (Wright *et al.*, 1997), separates the ciliates with postciliodesmata *sensu stricto* from all other ciliates. This branch corresponds to the subphylum POSTCILIODESMATOPHORA Gerassimova & Seravin, 1976 *sensu* Small & Lynn, 1985 with the hypotrich ciliates removed (Lynn, 1996). In our revised classification we are retaining this subphylum, but including only the classes KARYORELICTEA and HETEROTRICHEA (Table 3). The heterotrichs had been raised to class status by de Puytorac *et al.* (1993) (Table 2). Karyorelictean ciliates do not have dividing macronuclei (Raikov, 1969) while heterotrichs and all other ciliates do. However, heterotrichs apparently divide their macronuclei using extramacronuclear microtubules (e.g., *Blepharisma* - Figs. 3.38, 3.39 in Jenkins, 1973; *Stentor* - Fig. 18a in Diener *et al.*, 1983), while all other ciliates seemingly divide the macronucleus by intramacronuclear microtubules (Lynn, 1996; Orías, 1991). Lynn (1996) used the character of intramacronuclear microtubules to define the subphylum INTRAMACRONUCLEATA Lynn, 1996, which we have used in our revised classification (Table 3).

The suggestion that macronuclear division has arisen separately twice during the evolution of ciliates is not unreasonable, considering that at least two kinds of nuclear division using both extranuclear and intranuclear microtubules also occur in the dinoflagellates (Perret *et al.*, 1991), the sister clade to the ciliates (Fig. 1). Could it be that the genes for expression of both extranuclear and intranuclear microtubules resided in the common ancestor of the alveolates? Or is the evolution of extranuclear microtubules for division a convergent event in some ciliates and dinoflagellates?

#### How Many Superclasses?

Small and Lynn (1981, 1985) did not establish superclasses. However, de Puytorac *et al.* (1993) have recognized five superclasses: the superclasses POSTCILIODESMATOPHORA Gerassimova & Seravin, 1976,



SPIROTRICHA Butschli, 1889, and TRANSVERSALA de Puytorac *et al.*, 1993 in the subphylum TUBULICORTICATA; and the superclasses CILIOSTOMATOPHORA de Puytorac *et al.*, 1993 and MEMBRANELLOPHORA Jankowski, 1975 in the subphylum EPIPLASMATA (Table 2).

The superclass POSTCILIODESMATOPHORA *sensu de Puytorac et al.*, 1993 includes essentially the same taxa as our revised subphylum of the same name (cf. Tables 2, 3). However, on the basis of SSrRNA gene sequences (Hammerschmidt *et al.*, 1996), we have removed the monotypic subclass Protocruziidia to our class SPIROTRICHEA (Table 3). The superclass SPIROTRICHA *sensu de Puytorac et al.*, 1993 includes essentially the same taxa as our revised class SPIROTRICHEA (cf. Tables 2, 3). The superclass TRANSVERSALA *sensu de Puytorac et al.*, 1993 unites two classes, the PLAGIOPYLEA and the COLPODEA, whose somatic kinetics bear very little resemblance to each other (Lynn, 1991). De Puytorac *et al.* characterize this superclass as having transverse microtubular ribbons that are always longitudinally oriented along the length of kinetics (de Puytorac *et al.*, 1993). However, this ignores the very different origins within the kinetic of the transverse microtubular ribbons in the two classes (cf. Figs. 2a-c, Sa in Lynn, 1991), it simplifies the complexity of interactions among the sets of transverse microtubular ribbons in the cortex of members of the COLPODEA (Lynn, 1976b), and it ignores the more or less longitudinal orientation of the T1 set of transverse microtubules of the members of the LITOSTOMATEA (Lynn, 1991). Finally, SSrRNA gene sequences strongly suggest that members of the classes COLPODEA and PLAGIOPYLEA, although being members of the same major clade are not sister taxa (Fig. 1).

The superclass CILIOSTOMATOPHORA *sensu de Puytorac et al.*, 1993 only includes the class PHYLLOPHARYNGEA, which is composed of the same taxa in the classification of de Puytorac *et al.* (1993) and our revised classification (cf. Tables 2,3). Finally, the superclass MEMBRANELLOPHORA *sensu de Puytorac et al.*, 1993 includes the classes NASSOPHOREA and OLIGOHYMENOPHOREA (Table 2). This superclass includes ciliates with oral structures that include adoral polykinetids that can be reduced to three in number and a paroral on the right side, which can be circular when the oral region is apical. This last qualification of the description is necessary because de Puytorac *et al.* (1993) included the prostomate ciliates as a subclass within the class NASSOPHOREA (Table 2). Phylogenies derived from SSrRNA gene sequences clearly demonstrate a relationship between the classes NASSOPHOREA and OLIGOHYMENOPHOREA (Fig. 1; Stechmann *et al.*, 1997). However, they are rarely sister taxa (Wright and Lynn, unpubl. data), and typically members of the class PLAGIOPYLEA are more closely related to the oligohymenophoreans than are the nassophoreans. Thus, we are not convinced that these superclasses are meaningful conceptions.

We do not believe it is necessary to establish superclasses within our subphylum POSTCILIODESMATOPHORA (Table 3). Nevertheless, the phylogenies derived from distance analysis of SSrRNA gene sequences clearly demonstrate substructure within the subphylum INTRAMACRONUCLEATA (Fig. 1). Typically, the classes LITOSTOMATEA, SPIROTRICHEA, armophorids, and clevelandellids are in one clade (Fig. 1; Affa'a *et al.*, unpubl. data) while the classes PHYLLOPHARYNGEA, NASSOPHOREA, COLPODEA, PROSTOMATEA, PLAGIOPYLEA, and OLIGOHYMENOPHOREA are in the other clade (Fig. 1; Stechmann *et al.*, 1997). However, this topology is not supported by parsimony (Wright *et al.*, 1997). Although we believe future research will uncover characters that can be used to unambiguously describe superclasses within the subphylum INTRAMACRONUCLEATA, it is premature at this time to recognize any superclasses. We answer the question posed in the title to this section - "Perhaps two." - but we are not sure how many, and we do not know how to distinguish them.

#### How Many Classes?

The classification of Small and Lynn (1981, 1985) increased the number of classes of ciliates from the three proposed by de Puytorac *et al.* (1974) and Corliss (1979) to eight (Table 1). De Puytorac *et al.* (1993) have increased this number to 11 by recognizing the heterotrichs, oligotrichs, plagiopylians, and vestibuliferians as classes and submerging the prostomates within the class NASSOPHOREA (Table 2). Differences in the kinetic (Eisler, 1989; Lynn, 1985, 1991), stomatogenesis (Eisler, 1989; Hultenlauch and Bardele, 1987), and SSrRNA gene sequences (Stechmann *et al.*, 1997) between nassophoreans and prostomateans argue against uniting them within the same class. Thus, we cannot accept reducing the rank of the class PROSTOMATEA *sensu* Small and Lynn, 1985. But what about the other classes?

We concur with de Puytorac *et al.* (1993) that the heterotrichs should be elevated to class rank (cf. Tables 2, 3). The sequence divergence between heterotrichs and spirotrichs is very great (Fig. 1; Baroin-Tourancheau *et al.*, 1995; Hammerschmidt *et al.*, 1996; Hirt *et al.*, 1995), and their modes of stomatogenesis are very different (Foissner, 1996). Since there is only one published analysis of a partial SSrRNA sequence of *Halteria* derived from reverse

transcriptase sequencing (Lynn and Sogin, 1988), we believe there are too few data to warrant elevating the oligotrichs to class rank. Moreover, there are considerable similarities in the stomatogenetic patterns between some oligotrichs and stichotrichs (Foissner, 1996), and substantial differences between stomatogenesis of some taxa currently considered oligotrichs (Dale and Lynn, unpubl. data; Petz and Foissner, 1992). It may be that the oligotrichs are polyphyletic.

Small and Lynn (1985) recognized the subclass Plagiopylia as a new one within the class OLIGOHYMENOPHOREA. We agree that recognition of class rank for the plagiopylians by de Puytorac *et al.* (1993) can be justified on two grounds: 1) somatic kinetic differences are substantial between plagiopylians and all other ciliates (Berger and Lynn, 1984; Lynn, 1991), even the oligohymenophoreans with which Small and Lynn (1985) united them originally; and 2) phylogenies derived from SSrRNA gene sequences consistently demonstrate a significant genetic divergence between plagiopylians and other oligohymenophoreans (Fig.1). However, a careful analysis of the stomatogenesis of plagiopyleans, especially using electron microscopy, may reveal oligohymenophorean affinities that could argue for placing them again as a subclass within the class OLIGOHYMENOPHOREA.

Finally, we cannot agree with de Puytorac *et al.* (1993) that elevation of the vestibuliferians to class rank is warranted. We prefer to refer to this clade by the old Bütschlian moniker, Trichostomatia (cf. Tables 2, 3). The trichostomatians *sensu* Small and Lynn (= vestibulifereans *sensu* de Puytorac *et al.*) and haptorians share virtually identical somatic kinetic patterns (Lynn, 1981, 1991). This varies only in the entodiniomorphids where Lynn (1991) has interpreted the appearance of a transient microtubule during somatic kinetic replication (see Furness and Butler, 1986) to be the homologue of the T2 transverse microtubular ribbon. Moreover, extensive analyses of litostome SSrRNA gene sequences consistently group the haptorians and trichostomes whose genetic divergence, at least on this marker, is significantly less than genetic divergences within the classes SPIROTRICHEA and OLIGOHYMENOPHOREA. for example (Fig. 1; Wright and Lynn, 1997).

Thus, our revised classification (Table 3) differs from Small and Lynn (1981, 1985) in recognizing two new classes, the HETEROTRICHEA and PLAGIOPYLEA, bringing the total to 10. These are clades that were recognized at subclass rank previously so their descriptions remain unchanged. Affa'a *et al.* (unpubl. data) have preliminary evidence from SSrRNA sequences that armophorids and clevelandellids form a clade that is the sister taxon to the Litostomatea. However, until this is verified and until we have an understanding of what shared derived characters unite these two groups of ciliates, we have left these orders along with odontostomatids and phacodiniids as *sedis mutabilis* in the subphylum INTRAMACRONUCLEATA (Table 3).

Table 3. A Revised Classification of the Phylum Ciliophora

Phylum CILIPHORA Doflein, 1901

Subphylum *POSTCILIODESMATOPHORA* Gerassimova & Seravin. 1976

Class KARYORELICTEA Corliss. 1974

Order Protostomatida Small & Lynn, 1985

Order Loxodida Jankowski in Small & Lynn, 1985

Order Protoheterotrichida Nouzarède, 1977

Class HETEROTRICHEA Stein, 1859\*

Order Heterotrichida Stein. 1859

Order Licnophorida Corliss. 1957

Subphylum *INTRAMACRONUCLEATA* Lynn, 1996

Class SPIROTRICHEA Bütschli. 1889

Subclass Protocruziidia de Puytorac *et al.*, 1984\*

Order Protocruziida Jankowski in Small & Lynn, 1985

Subclass Hypotrichia Stein, 1859

Order Kiiitrichida Nozawa. 1941 \*

Order Euplotida Small y Lynn. 1985

Subclass Choreotrichia Small & Lynn 1985\*

Order Tintinnida Kofoid & Campbell, 1929\*

Order Choreotrichida Small & Lynn, 1985

Subclass Stichotrichia Small & Lynn, 1985

- Order Plagiotomida Albaret, 1974
- Order Stichotrichida Fauré-Fremiet. 1961
- Order Urostylida Jankowski, 1979\*
- Order Sporadotrichida Fauré-Fremiet, 1961 \*
- Subclass Oligotrichia Bütschli, 1887\*
- Order Halteriida Petz & Foissner. 1992\*
- Order Strombidiida Petz & Foissner, 1992\*

*Sedis mutabilis* in the Subphylum Intramacronucleata

- Order Armophorida Jankowski. 1964
- Order Clevelandellida de Puytorac & Grain, 1976
- Order Phacodiniida Small & Lynn, 1985
- Order Odontostomatida Sawaya, 1940

Class LITOSTOMATEA Small y Lynn, 1981

- Subclass Haptoria Corliss. 1974
  - Order Haptorida Corliss, 1974
  - Order Pleurostomatida Schewiakoff 1896
  - Order Cyclotrichida Jankowski, 1980\*
- Subclass Trichostomatia Bütschli, 1889
  - Order Vestibuliferida de Puytorac et al., 1974
  - Order Entodiniomorphida Reichenow in Doflein & Reichenow, 1929

Class PHYLLOPHARYNGEA de Puytorac *et al.*, 1974

- Subclass Phyllopharyngia de Puytorac et al., 1974
  - Order Chlamyodontida Deroux, 1976\*
  - Order Dysteriida Deroux, 1976\*
- Subclass Rhynchodia Chatton & Lwoff 1939\*
  - Order Rhynchodida Chatton & Lwoff, 1939
  - Order Hypocomatida Deroux, 1976\*
- Subclass Chonotrichia Wallengren, 1895
  - Order Exogemmida Jankowski, 1972
  - Order Cryptogemmida Jankowski, 1975
- Subclass Suctoria Claparede & Lachmann, 1858
  - Order Exogenida Collin, 1912
  - Order Endogenida Collin, 1912
  - Order Evaginogenida Jankowski in Corliss, 1979

Class NASSOPHOREA Small & Lynn, 1981

- Order Synhymeniida de Puytorac et al., 1974
- Order Nassulida Jankowski, 1967
- Order Microthoracida Jankowski, 1967

Class COLPODEA Small & Lynn, 1981

- Order Cyrtolophosidida Foissner, 1978
- Order Bryometopida Foissner, 1985
- Order Bryophryida de Puytorac et al., 1979
- Order Bursariomorphida Fernández-Galiano, 1978
- Order Colpodida de Puytorac et al, 1974
- Order Sorogenida Foissner, 1985

Class PROSTOMATEA Schewiakoff, 1896

- Order Prostomatida Schewiakoff, 1896
- Order Prorodontida Corliss, 1974

Class PLAGIOPYLEA Small & Lynn, 1985

- Order Plagiopylida Small & Lynn, 1985

Class OLIGOHYMENOPHOREA de Puytorac *et al.*, 1974

- Subclass Peniculia Fauré-Fremiet in Corliss, 1956\*
- Order Peniculida Fauré-Fremiet in Corliss, 1956

- Subclass Scuticociliatia Small, 1967
  - Order Philasterida Small, 1967\*
  - Order Pleuronematida Fauré-Fremiet in Corliss, 1956\*
  - Order Thigmatrichida Chatton & Lwoff, 1922\*
- Subclass Hymenostomatia Delage & Herouard, 1896
  - Order Hymenostomatida Delage & Herouard, 1896
- Subclass Apostomatia Chatton & Lwoff 1928
  - Order Apostomatida Chatton & Lwoff, 1928
  - Order Astomatophorida Jankowski, 1966
  - Order Pilisuctorida Jankowski, 1966
- Subclass Peritrichia Stein, 1859
  - Order Sessilida Kahl, 1933
  - Order Mobilida Kahl, 1933
- Subclass Astomatia Schewiakoff 1896
  - Order Astomatida Schewiakoff 1896

\* Newly elevated or recognized taxa compared to Small and Lynn (1981, 1985). See Table 1.

### Subclasses and Orders

De Puytorac *et al.* (1993) have elevated a considerable number of taxa to subclass and ordinal rank. Their scheme currently has 25 subclasses and 70 orders (Table 2). It is beyond the scope of this paper to analyze in detail the justifications for these taxa. Comparison of our scheme (Table 3) with that of de Puytorac *et al.* (Table 2) will demonstrate considerable agreement in the basic groups or clades despite differences in rank. In this section, we will focus on a comparison of Small and Lynn (1981, 1985) and our revised scheme (cf. Tables 1, 3).

Small and Lynn (1981, 1985) established 15 subclasses and 48 orders while our revised scheme has 17 subclasses and 57 orders. In agreement with de Puytorac *et al.* (1993), we have elevated the rank of five orders, the Protocruziida, Oligotrichida, Rhynchodida, Peniculida, and Scuticociliatida to subclass (cf. Tables 2,3). In elevating the subclasses Heterotrichia and Plagiopylia to class rank, we have eliminated the subclasses Heterotrichia and Plagiopylia. Transfer of the subclass Hypotrichia to the class SPIROTRICHEA has eliminated the subclass Nassophoria. This explains the increase from 15 to 17 subclasses.

The explanation of what we have done with orders is slightly more complicated. In summary, we have eliminated 6 orders from Small and Lynn (1981, 1985) (Table 1) and added 15 (Table 3). We have eliminated the order Peritromida and placed the family Peritromidae in the order Heterotrichida on the basis of recent protargol stains by Song and Wilbert (1997) that demonstrated *Peritromus* to have postciliodesmata (Figs. 38, 40 in Song and Wilbert, 1997). Following de Puytorac *et al.*, 1993 (Table 2), we have included the new order Kiiitrichida in our subclass Hypotrichia (Table 3). Within the subclass Stichotrichia, we have elevated to orders the Urostylida and Sporadotrichida (cf. Tables 1, 3). In recognizing the subclass Oligotrichia, we have eliminated the order Oligotrichida, but recognized three additional orders, the orders Halteriida and Strombidiida in the subclass Oligotrichia and the order Tintinnida in the subclass Choreotrichia (Table 3), following de Puytorac *et al.* (1993). In the class LITOSTOMATEA, we have eliminated the order Pharyngophorida and added the order Cyclotrichida (Table 3). In the subclass Phyllopharyngia, we have eliminated the order Cyrtophorida and added the orders Chlamyodontida and Dysteriida (Table 3). In accepting the subclass Rhynchodia, we have added the order Hypocomatida (Table 3). We have eliminated the order Propeniculida, and placed the Pseudomicrothoracidae in the order Microthoracida. Finally, in elevating the scuticociliates to subclass, we have elevated the included suborders to orders, recognizing the Philasterida, Pleuronematida, and Thigmatrichida.

### Conclusions

We are gratified to see that ciliatologists in France and in North America are converging on similar schemes of classification for this important phylum of protists. Major differences between schemes (cf. Tables 2, 3) rest mainly at the levels of higher taxonomic ranks and lower taxonomic ranks, that is, the subphyla and orders. As additional data, especially from gene sequences, are obtained, we are confident that a global consensus will emerge as the new millennium arrives!!

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