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α-Tubulin of *Histriculus cavicola* (Ciliophora; Hypotrichea)

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Summary

An α-tubulin gene fragment amplified by PCR from the hypotrichous ciliate *Histriculus cavicola* has been sequenced. This fragment, 1,182 bp long, contains an in-frame "stop" codon (UAA), which in other hypotrichous species codes for a glutamine residue. The comparison of the α-tubulin genes from several ciliates classes have revealed amino acid positions which could serve to distinguish these taxonomic groups.

Key words: *Histriculus cavicola*, α-tubulin, UAA codon, phylogeny, hypotrichs

Resumen

Se ha secuenciado un fragmento de un gen de la α-tubulina, amplificado por PCR, del ciliado hipotrico *Histriculus cavicola*. Este fragmento, de 1182 pb, contiene un codón de terminación (UAA) en la fase de lectura, que en otros ciliados hipotricos codifica la glutamina. La comparación de los genes de la α-tubulina de varias clases de ciliados ha mostrado que algunas posiciones de aminoácidos pueden servir para distinguir estos grupos taxonómicos.

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Introduction

Microtubules are present in all eukaryotic cells as a major component of the cytoskeleton. They are involved in many cellular functions, such as motility, cell division, location of the Golgi and endoplasmic reticulum (ER), and establishment of cell polarity (8).

The major protein of microtubules is tubulin, a heterodimer of two distinct polypeptides designated as α and β. Each subunit has a molecular mass of about 50,000 Da and consists of different isotypes which are highly conserved in eukaryotic evolution. Both are generally post-translationally modified (18).

In higher eukaryotes, α- and β-tubulins are encoded by complex gene families. Tubulin heterogeneity therefore can be attributed both to primary genetic diversity and to a variety of post-translational modifications (5, 6, 12, 13).

Ciliates have a large variety of microtubular networks with functional and morphological differences. At least 13 distinct microtubular arrays have been described in *Paramecium* (7). In spite of their microtubular diversity, both α-tubulins and β-tubulins of ciliates are encoded by a small number of strikingly similar genes. In fact, most differences in the properties of the diverse microtubular arrays can be attributed to post-translational modifications and/or to interactions with accesory proteins (4).

Genes coding for α-tubulin have been sequenced in fifteen species belonging to six classes of the phylum Ciliophora: *Stylonychia lemnae*, *Oxytricha granulifera*, *Euplotes octocarinatus*, *Euplotes vannus*, *Euplotes aediculatus* (Class Hypotrichea), *Tetrahymena pyriformis*, *Tetrahymena thermophila*, *Frontonia* sp. (Class Oligohymenophorea), *Zosterograptus* sp. (Class Nassophorea), *Stentor coeruleus*, *Condylostoma magnum* (Class Heterotrichea), *Spathidium* sp., *Epidinium* sp., *Entodinium* sp. (Class Litostomatea) and *Loxodes striatus* (Class Karyorelictea).

We sequenced a 1,182 bp fragment of an α-tubulin gene from *Histriculus cavicola*, a hypotrichous ciliate that forms resting cysts under adverse conditions. This sequence contains a canonical in-frame "stop" codon (UAA) that instead codes for a glutamine residue. Comparison of the amino acid sequences of *Histriculus* α-tubulin with α-tubulins from other ciliates indicates that some amino acids are characteristic for each of the ciliate classes studied. In addition, our results support taxonomic relationships based on rRNA sequences.

Material and methods

H. cavicola was grown at a temperature of 20°C in Pringsheim's solution and fed with the green algae *Chlorogonium* sp. DNA was isolated from starved vegetative cells following conventional methods (14). *Histriculus* α-tubulin gene was amplified with Taq polymerase (Boehringer) from genomic DNA using degenerate oligonucleotides as primers. The sense and antisense primers, based on the conserved CLEHGIQ (position 25–31 respect to *Tetrahymena pyriformis* published α-tubulin, standard and referred gene in this paper) and MEEGEF (position 413–418) amino acid sequences, were:

5' TGYYTKGAGCAYGGTATYCAACC 3'
5' SAGAAYTCTCCTTCYTCCAT 3'

PCR amplification conditions were as follows: denaturation at 94°C for 30 s, annealing at 55°C for 30 s and polymerization at 72°C for 1 min (35 cycles). The amplified product was isolated and blunt-end ligated into the *Eco*RV site of the pBluescript II KS vector (Stratagene, La Jolla, CA, USA). The insert was sequenced using the DIG-Taq sequencing kit (Boehringer) and the direct blotting electrophoresis (MWG-Biotech). The sequence is in the EMBL database, with the accession number Y10035.

The amino acid sequences were aligned with the following α-tubulin sequences taken from the GenEMBL database (with their respective accession numbers): *Stylonychia lemnae* (X01746 and X12365); *Oxytricha granulifera* (Z11763); *Euplotes octocarinatus* (X69466); *Euplotes vannus* (Z11769); *Euplotes aediculatus* (Z49851); *Tetrahymena pyriformis* (X12767); *Tetrahymena thermophila* (M86723); *Zosterograptus* sp. (Z49855); *Stentor coeruleus* (Z49853); *Condylostoma magnum* (Z49850); *Spathidium* sp. (Z49848); *Epidinium* sp. (Z49856); *Entodinium* sp. (Z49849); *Loxodes striatus* (Z49852). The sequence of *Frontonia* sp. was kindly provided by Anne Baroin-Tourancheau (Orsay, France).

Computer analyses were made through the Wisconsin Sequence Analysis Package (Genetic Computer Group), version 8.1. The alignment was performed using PILEUP program.

Results and discussion

PCR reactions gave a single band of about 1.1 kb. No bands were observed in those reactions lacking template. The amplified DNA was cloned and several positive selected. Among the positive clones, one was completely sequenced in both strands. The fragment contained 1,182 nucleotides.

The nucleotide and deduced amino acid sequence is presented in Fig. 1. This DNA fragment contains most (90%) of the coding region of a standard α-tubulin gene, e.g., *Tetrahymena pyriformis*, and does not contain introns.

Four putative GTP binding sites (15) were present: VPRCVFLDLEP (aa. 62–72) and RFDGAL (aa. 243–248), both involved in base binding; GGGTGSG (aa. 142–148), implicated in ribose binding; and AVVEPYN (aa. 180–186), involved in phosphate binding. The fragment has a lysine residue located at position 40, which may serve as a substrate for a post-translational acetylation (16).

The sequence contains a UAA codon in frame (nucleotide 526–528) that, as described in other hypotrichs (2), is used as a sense codon for a glutamine residue.

The G+C content of the fragment was 49.6%. The codon usage for this gene fragment is showed in Table 1. Of the 61 existing codons, only 38 are used. Three of them are used once, and six are used twice. In addition to methionine and tryptophan, five amino acids are codified by only one codon (arg/AGA, asn/AAC, cys/TGC, phe/TTC, gln/CAA). The nucleotide third-position preference can be summarized as follows: C(44.5%)>T(23.3%)>A(21.7%)>G(10.5%). Of the codons used, 45% have A or T in the third position.

Codon bias correlates with the level of the gene expression in a wide range of organisms (3). In general, genes coding for highly expressed proteins show a strong bias for codons complementary to the major isoacceptor tRNA species.

Sequence analyses of additional *Histriculus* genes will be required to verify whether the bias in the α-tubulin gene is representative of the codon subset used to encode highly expressed *Histriculus* proteins.

The *Histriculus* gene fragment shows a great degree of sequence similarity with other ciliates. The nucleotide homology between this fragment (taking met-32 to gly-412) and other ciliate α-tubulins is shown in Table 2. The *Histriculus* fragment is most similar to other hypotrichous ciliate tubulin genes, e.g., *Oxytricha* (92.5%) and *Stylonychia* (93.9–93.4%). The percentage of identity with the euplotids ranges from 87.7% to 85.4%. In descending order of similarity, the

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tgcttggagcacggtatccaaccTGACGGTCAAATGCCATCAGACAAGACTATTGGTGGT  132
.......................D  G  Q  M  P  S  D  K  T  I  G  G
     GGTGATGATGCCTTCAACACTTTCTTCTCCGAAACTGGAGCTGGCAAGCACGTC  186
      G  D  D  A  F  N  T  F  F  S  E  T  G  A  G  K  H  V
     CCAAGATGCGTCTTCCTCGATCTCGAGCCAACCGTTATCGATGAAGTTAGAACC  240
      P  R  C  V  F  L  D  L  E  P  T  V  I  D  E  V  R  T
     GGTACCTACAGACAACTCTTCCACCCTGAGCAACTCATCTCAGGTAAAGAAGAT  294
      G  T  Y  R  Q  L  F  H  P  E  Q  L  I  S  G  K  E  D
     GCTGCCAACAACTTCGCCAGAGGTCACTACACCATCGGTAAAGAAATCGTCGAT  348
      A  A  N  N  F  A  R  G  H  Y  T  I  G  K  E  I  V  D
     CTCTGCCTCGATAGAATCAGAAAGCTCGCTGATCAATGCACTGGTCTCCAAGGT  402
      L  C  L  D  R  I  R  K  L  A  D  Q  C  T  G  L  Q  G
     TTCCTCGTCTTCAACTCAGTCGGTGGTGGTACTGGATCCGGTCTCGGTTCACTC  456
      F  L  V  F  N  S  V  G  G  G  T  G  S  G  L  G  S  L
     CTCCTCGAAAGACTCTCCGTCGATTACGGTAAGAAGTCAAAGCTCGGTTTCACC  510
      L  L  E  R  L  S  V  D  Y  G  K  K  S  K  L  G  F  T
     GTCTACCCATCACCATAAGTCTCAACTGCCGTCGTTGAGCCATACAACTCAGTG  564
      V  Y  P  S  P  Q  V  S  T  A  V  V  E  P  Y  N  S  V
     CTCTCAACTCACTCACTCCTCGAACATACTGATGTTGCTGTTATGCTCGATAAC  618
      L  S  T  H  S  L  L  E  H  T  D  V  A  V  M  L  D  N
     GAAGCCGTCTACGATATCTGCAGAAGAAACCTCGATATTGAGAGACCAACCTAC  672
      E  A  V  Y  D  I  C  R  R  N  L  D  I  E  R  P  T  Y
     ACCAACTTGAACAGACTCATCGCTCAAGTTATCTCATCATTGACTGCCTCACTC  726
      T  N  L  N  R  L  I  A  Q  V  I  S  S  L  T  A  S  L
     AGATTCGATGGTGCCTTGAACGTCGATGTTACTGAATTCCAAACCAACTTGGTC  780
      R  F  D  G  A  L  N  V  D  V  T  E  F  Q  T  N  L  V
     CCATATCCAAGAATCCATTTCATGTTGTCATCATACGCCCCAGTCATCTCAGCT  834
      P  Y  P  R  I  H  F  M  L  S  S  Y  A  P  V  I  S  A
     GAGAAGGCTTATCACGAACAACTCTCAGTTGCTGAAATCACCAACTCAGCTTTC  888
      E  K  A  Y  H  E  Q  L  S  V  A  E  I  T  N  S  A  F
     GAGCCCGCTTCCATGATGGCCAAGGTCGACCCAAGACACGGTAAATATATGGCT  942
      E  P  A  S  M  M  A  K  V  D  P  R  H  G  K  Y  M  A
     TGCTGCCTCATGTACAGAGGTGATGTCGTCCCCAAGGATGTCAACGCTGCCGTC  996
      C  C  L  M  Y  R  G  D  V  V  P  K  D  V  N  A  A  V
     GCCACCATCAAGACCAAGAGAACCATCCAATTCGTCGACTGGTGCCCAACTGGC 1050
      A  T  I  K  T  K  R  T  I  Q  F  V  D  W  C  P  T  G
     TTCAAGTGCGGTATCAACTATCAACCACCAACAGTCGTCCCAGGTGGTGATCTC 1104
      F  K  C  G  I  N  Y  Q  P  P  T  V  V  P  G  G  D  L
     GCCAAGGTTATGAGAGCCGTCTGCATGATCTCCAACTCAACTGCCATCGCTGAG 1158
      A  K  V  M  R  A  V  C  M  I  S  N  S  T  A  I  A  E
     GTCTTCTCAAGAATCGATCACAAGTTCGATCTCATGTACGCCAAGAGAGCCTTC 1212
      V  F  S  R  I  D  H  K  F  D  L  M  Y  A  K  R  A  F
     GTCCACTGGTACGTCGGTGAAGGTatggaggaaggagaattctg           1256
      V  H  W  Y  V  G  E  G  ......................
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FIG. 1. Nucleotide and predicted amino acid sequence (one code letter) of the amplified fragment from *Histriculus* α–tubulin. The sequence of the synthetic primers used for PCR are underlined. The in-frame UAA "stop" codon and the corresponding gln are in bold letters. The GTP-binding domains are in bold and underlined. The numbering scheme refers to the nucleotides of the published *Tetrahymena pyriformis* α-tubulin gene.

TABLE 1. Codon usage of the amplified fragment of *Histriculus* α-tubulin. Underlined codons denote that they are the most frequently or nearly equally used for a given amino acid. The numbers are the total number of occurrences of each codon. The in-frame "stop" codon is in bold letter

Codon	aa.	No.									
TTT	phe	–	TCT	ser	–	TAT	tyr	4	TGT	cys	–
TTC	phe	19	TCC	ser	5	TAC	tyr	11	TGC	cys	10
TTA	leu	–	TCA	ser	20	**TAA**	**gln**	**1**	TGA	OPA	–
TTG	leu	6	TCG	ser	–	TAG	AMB	–	TGG	trp	2
CTT	leu	–	CCT	pro	2	CAT	his	2	CGT	arg	–
CTC	leu	26	CCC	pro	2	CAC	his	9	CGC	arg	–
CTA	leu	–	CCA	pro	15	CAA	gln	11	CGA	arg	–
CTG	leu	–	CCG	pro	–	CAG	gln	–	CGG	arg	–
ATT	ile	2	ACT	thr	12	AAT	asn	–	AGT	ser	–
ATC	ile	18	ACC	thr	12	AAC	asn	15	AGC	ser	–
ATA	ile	–	ACA	thr	1	AAA	lys	3	AGA	arg	19
ATG	met	11	ACG	thr	–	AAG	lys	15	AGG	arg	–
GTT	val	9	GCT	ala	13	GAT	asp	20	GGT	gly	26
GTC	val	25	GCC	ala	16	GAC	asp	4	GGC	gly	2
GTA	val	–	GCA	ala	–	GAA	glu	13	GGA	gly	3
GTG	val	1	GCG	ala	–	GAG	glu	9	GGG	gly	–

percentage of identities are: *Tetrahymena*, 86.7–86%; *Frontonia*, 86.5%; *Stentor*, 85%; *Zosterograptus*, 81.4%; *Loxodes*, 78.8%; litostomes (*Epidinium*, *Entodinium* and *Spathidium*), 78.8–76.9%; and *Condylostoma*, 77.9%.

As expected, nucleotide sequence homology was lower than the corresponding amino acid sequence homology (Table 2). Percentages of similarity between the deduced amino acid sequence of α-tubulin of *Histriculus* and those of the ciliates listed above ranged from 99.5% (*Stylonychia*) to 93.4% (*Spathidium*).

The alignment of the *Histriculus* α-tubulin amino acid sequence with those of other ciliates reveals some interesting points (Fig. 2). Several traditional taxonomic groups differ in the identity of amino acids present at a few key positions in the α-tubulin protein sequence. Table 3 summarizes the characteristic amino acid substitutions at several positions in the α-tubulin of litostomes, oligohymenophorans, pseudohypotrichs (Euplotia) and euhypotrichs (Oxytrichia).

The litostomes and the euplotids contain nine and five characteristic amino acids, respectively. These amino acid changes strongly support the uniquely derived status of these clades. The euhypotrichs show two characteristic amino acids and their α-tubulin sequences contain at the position 526–528 an in-frame UAA codon which codifies for glutamine 176. The oligohymenophorans contain asparagine at position 300 and serine at position 316.

Our sequence analysis indicates that a deep split exits between pseudohypotrichs, such as *Euplotes*, and the euhypotrichs, such as *Histriculus*, *Stylonychia* and *Oxytricha*. The data agree with the results of Baroin-Tourancheau et al. (1), who reported a large divergence be-

TABLE 2. Nucleotide (a) and amino acid (b) homology (% identity) between the *Histriculus cavicola* α-tubulin and the α-tubulin from other ciliates

Ciliate	a	b
Class Hypotrichea		
Subclass Oxytrichia (Euhypotrichs)		
Stylonychia lemnae 1	93.9	99.5
Stylonychia lemnae 2	93.4	98.2
Oxytrichia granulifera	92.5	97.0
Subclass Euplotia (Pseudohypotrichs)		
Euplotes octocarinatus	87.7	96.8
Euplotes aediculatus	87.5	95.8
Euplotes vannus	85.4	94.7
Class Oligohymenophorea		
Tetrahymena pyriformis	86.7	96.3
Tetrahymena thermophila	86.0	96.0
Frontonia sp.	86.5	95.8
Class Heterotrichea		
Stentor coeruleus	85.0	95.0
Condylostoma magnum	77.9	97.4
Class Nassophorea		
Zosterograptus sp.	81.4	96.0
Class Karyorelictea		
Loxodes striatus	78.8	95.8
Class Litostomatea		
Epidinium sp.	78.8	93.7
Entodinium sp.	77.6	93.5
Spathidium sp.	76.9	93.4

tween *Euplotes* and the euhypotrichs based on rRNA sequence analysis.

Surprisingly, the single representative of nassophoreans, *Zosterograptus*, contains, as do the euhypotrichs, glutamine at position 128 and leucine at position 317. Additional α-tubulin sequences are required to test the association of the nassophoreans with the euhypotrichs.

In the heterotrichs (*Stentor*, *Condylostoma* and *Blepharisma* [data not shown]) an asparagine residue is replaced by a glutamine at position 215. The same amino acid substitution is also observed in the karyorelictid *Loxodes* and in the litostomes. Recently, both Baroin-Tourancheau et al. (1) and Hammerschmidt et al. (10), used rRNA sequences to demostrate that the karyorelicteans are the sister group of the heterotrichs.

To sum up, the α-tubulin genes are good markers for the inference of relationships among ciliates. The comparative analysis of α-tubulin sequences in general supports classical taxonomic groups based on ultrastructural and other morphological characters. In addition, the analysis reinforces phylogenetic relationships derived from comparisons of rRNA sequences, for example the split between euplotids and euhypotrichs, and the similarity between *Loxodes* and the heterotrichs.

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                           40         50         60         70         80         90
H. cavicola          DGQMPSDK TIGGGDDAFN TFFSETGAGK HVPRCVFLDL EPTVIDEVRT GTYRQLFHPE
O.granulifera        -------- ---------- ---------- ---------- ----D----- ----------
S. lemnae (1)        -------- ---------- --------E- ---------- ---------- ----------
S. lemnae (2)        ------N- ---------- -------T-- ---------- ---------- ----------
Zosterograptus sp.   -------- ---------T ---------- Y-----M--- ---------- ----------
E. vannus            -------- ---------- ---------- ----A-LV-- ----C--I-- ----------
E. aediculatus       -------- ---------- ---------- ----A----- ----C----- ----------
E. octocarinatus     -------- ---------- ---------- ----A----- ----C----- ----------
Entodinium sp.       -------- ---------- ---------- ----S----- ----V----- ----------
Epidinium sp.        -------- ---------- ---------- ----S----- ---------- ----------
Spathidium sp.       -------- ---------- ---------- ---------- ----V----- ----------
T. pyriformis        -------- ---------- ---------- ----A----- ---------- ----------
T. thermophila       -------- ---------- ---------- ----A----- ---------- ----------
S. coeruleus         -------- ---------- ---------- ----A----- ---------- ----------
C. magnum            -------- ---------- ---------- ----A----- ----V----- --I-------
L. striatus          -------- ---------- ---------- ----S----- ---------- ----------
                          100        110        120        130        140        150
H. cavicola        QLISGKEDAA NNFARGHYTI GKEIVDLCLD RIRKLADQCT GLQGFLVFNS VGGGTGSGLG
O. granulifera     ---------- ---------- ---------- ---------- ---------- ----------
S. lemnae (1)      ---------- ---------- ---------- ---------- ---------- ----------
S. lemnae (2)      ---------- ---------- ---------- G--------- ---------- ----------
Zosterograptus sp. ---------- ---------- ---------- ---------P ---------- ----------
E. vannus          -I-------- ---------- -------V-- -------N-- -----IG-H- ----------
E. aediculatus     ---------- ---------- ---------- ---R---N-- -----IG-H- ----------
E. octocarinatus   ---------- ---------- ---------- -------N-- -----IG-H- ----------
Entodinium sp.     ---------- --Y---YC-- ----I--A-- -------N-- ---------A ----------
Epidinium sp.      ---------- --Y---YC-- ----I--A-- -------N-- ---------A ----------
Spathidium sp.     ---------- --Y---YC-- ----I--A-- -------N-- ---------A ----------
T. pyriformis      ---------- ---------- ---------- -------N-- ---------- ----------
T. thermophila     ---------- ---------- ---------- -------N-- ---------- ----------
S. coeruleus       ---T------ ---------- ---------- -------N-- ---------- ----------
C. magnum          ---------- ---------- ---------- -------N-- ---------- ----------
L. striatus        ---------- ---------- ---------- -------N-- ---------A ----------
                          160        170        180        190        200        210
H. cavicola        SLLLERLSVD YGKKSKLGFT VYPSPQVSTA VVEPYNSVLS THSLLEHTDV AVMLDNEAVY
O. granulifera     ---------- ---------- ---------- ---------- ---------- ----------
S. lemnae (1)      ---------- ---------- ---------- ---------- ---------- ----------
S. lemnae (2)      ---------- ---------- I--------- ---------- ---------- ----------
Zosterograptus sp. ---------- ---------- I--------- ---------- ---------- --------I-
E. vannus          ---------- ---------- I--------- ---------- ---------- ----------
E. aediculatus     ---------- -------T-- I--------- ---------- ---------- ----------
E. octocarinatus   ---------- -------T-- I--------- ---------- ---------- ----------
Entodinium sp.     ---------- ---------- ------I-N- -------I-- ---------- --------I-
Epidinium sp.      ---------- ---------- ------I-N- -------I-- ---------- --------I-
Spathidium sp.     ---------- ---------- I-----I-N- -------I-- ---------- --------I-
T. pyriformis      ---------- ---------- I--------- -------I-- ---------- --------I-
T. thermophila     ---------- ---------- I--------- -------I-- ---------- --------I-
S. coeruleus       ---------- ---------- I--------- ---------- ---------- ----------
C. magnum          ---------- ---------- IH-------- ---------- ---------- ----------
L. striatus        ---------- ---------- ---------- ---------- ---------- --------I-
                          220        230        240        250        260        270
H. cavicola        DICRRNLDIE RPTYTNLNRL IAQVISSLTA SLRFDGALNV DVTEFQTNLV PYPRIHFMLS
O. granulifera     ---------- ---------- ---------- ---------- ---------- ----------
S. lemnae (1)      ---------- ---------- ---------- ---------- ---------- ---SVIIR--
S. lemnae (2)      ---------- ---------- ---------- ---------- ---------- ----------
Zosterograptus sp.---------- ---------- -S-------- ---------- -I-------- ----------
E. vannus          ---------- ---------- ---------- ---------- -I-------- ----------
E. aediculatus     ---------- ---------- ---------- ---------- -I-------- ----------
E. octocarinatus   ---------- ---------- ---------- ---------- ---------- ----------
Entodinium sp.     -----Q---- ---------- ---------- ---------- -M-------- ----------
Epidinium sp.      -----Q---- ---------- ---------- ---------- -M-V------ ----------
Spathidium sp.     -----Q---- ---------- ---------- ---------- ---V------ ----------
T. pyriformis      ---------- ---------- ---------- ---------- -I-------- ----------
T. thermophila     ---------- ---------- ---------- ---------- -I-------- ----------
S. coeruleus       -----Q---- ---------- ---------- ---------- ---------- ----------
C. magnum          -----Q---- ---------- ---------- ---------- ---------- ----------
L. striatus        -----Q---- ---------- ---------- ---------- ---------- ----------
```

FIG. 2. Comparison of the deduced amino acid sequence of *Histriculus* α-tubulin is shown on the upper line. Amino acid identities are dotted, while differences are denoted by the corresponding single letter, indicating the substituted amino acid. The numering refers to the corresponding deduced amino acid sequence of the published *Tetrahymena pyriformis* α-tubulin gene. (For complete names of the genera, see text.)

The structure of ciliate phylogeny has traditionally rested on ultrastructural features of the cortex and of somatic and oral ciliature. The absence of derived morphological characters that unite groups and the difficulty in distinguishing between ancestral and derived morphologies make this kind of phylogeny assessment somewhat speculative. Recently, phylogenetic relationships within the phylum have been investigated by using 18S (9, 11,17) and 28S rRNAs (1). We believe extensive analyses of the α-tubulin sequences of ciliates should lead to the construction of convincing molecular trees. These results can be compared to rRNA trees and to phylogenies inferred from traditional morphological features. This approach will test the congruencies/incongruencies between two molecular markers along with more classical approaches.

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                          280        290        300        310        320        330
H. cavicola        SYAPVISAEK AYHEQLSVAE ITNSAFEPAS MMKAVDPRHG KYMACCLMYR GDVVPKDVNA
O. granulifera     ---------- ---------- ---------- ----C----- ---------- ----------
S. lemnae (1)      ----STP--- ---------- ---------- ----C----- ---------- ----------
S. lemnae (2)      ---------- ---------- ---------- ----C----- ---------- ----------
Zosterograptus sp. ---------- ---------- ----VY---- ----C----- ---------- --A-------
E. vannus          --G------- ---------- ----S----- ----C----- ------M-F- ----------
E. aediculatus     ---------- ---------- ----S----- ----C----- ------M--- ---------D-
E. octocarinatus   ---------- ---------- ----S----- ----C----- ------M--- ----------
Entodinium sp.     --R-I----- ---------- ---------- ----C----- ------M--- ----------
Epidinium sp.      ----I----- --D------- ---------- ----C----- ------M--- ----------
Spathidium sp.     ----I----- ---------- ----S----- ----C----- ------M--- ----------
T. pyriformis      ----I----- ---------- ---------N ----C----- -----SM--- ----------
T. thermophila     ----I----- ---------- ---------N ----C----- -----SM--- ----------
S. coeruleus       ----I----- ---------- ---------- ----C----- ------M--- ----------
C. magnum          ----I----- ---------- ----T----- ----C----- ------M--- ----------
L. striatus        ----I----- ---------- ----V----- S-V-C----- ------M--- ----------
                          340        350        360        370        380        390
H. cavicola        AVATIKTKRT IQFVDWCPTG FKCGINYQPP TVVPGGDLAK VMRAVCMISN STAIAEVFSR
O. granulifera     ---------- ---------- ---------- ---------- ---------- ----------
S. lemnae (1)      ---------- ---------- ---------- ----S--P-- ---------- ----------
S. lemnae (2)      ---------- ---------- ---------- ----S----- ---------- ----------
Zosterograptus sp. ---------- ----N----- ---------- A--------- ---------- ----------
E. vannus          ---------- ------S--- --V------- ---------- ---------- ----------
E. aediculatus     ---------- ------S--- ---------- ---------- ---------- ----------
E. octocarinatus   ---------- ------S--- ---------- ---------- ---------- ----------
Entodinium sp.     S--------- ---------- ---------- ---------- ---------- ----------
Epidinium sp.      S--------- ---------- --------FL ---------- ---------- ----------
Spathidium sp.     ---------- ---------- ---------- ---------- ---------- ----------
T. pyriformis      SI-------- ---------- --V------S ---------- ---------- ----------
T. thermophila     SI-------- ---------- --V------- ---------- ---------- ----------
S. coeruleus       ---------- ---------- ---------- -------H-- ---------- ----------
C. magnum          ---------- ---------- ---------- ---------- ---------- ----------
L. striatus        S--------- ---------- ---------- ---------- ---------- T---------
                          400        412
H. cavicola        IDHKFDLMYA KRAFVHWYVG EG
O. granulifera     ---------- ---------- --
S. lemnae (1)      ---------- ---------- --
S. lemnae (2)      ---------- ---------- --
Zosterograptus sp. ---------- ---------- --
E. vannus          ---------- ---------- --
E. aediculatus     ---------- ---------- --
E. octocarinatus   ---------- ---------- --
Entodinium sp.     L--------T -------F-- --
Epidinium sp.      L--------T -------F-- --
Spathidium sp.     L--------T -------F-- --
T. pyriformis      L--------- ---------- --
T. thermophila     L--------- ---------- --
S. coeruleus       ---------- ---------- --
C. magnum          ---------- ---------- --
L. striatus        ---------- ---------- --
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FIG. 2. (*Continued.*)

TABLE 3. Characteristic amino acids in the α-tubulin of some ciliate groups. Characteristic amino acids and their position are written in bold. Amino acids inside the parenthesis refer to those normally found outside the group

Group	Species	Marker
Class Litostomatea	*Entodinium* sp. *Epidinium* sp. *Spathidium* sp.	**tyr-103** (phe); **tyr-107** (his) **cys-108** (tyr); **ile-115**(val) **ala-118** (cys/val); **ile-177**(val) **asn-179** (thr); **thr-400** (ala) **phe-408** (tyr)
Subclass Euplotia (Pseudohypotrichs)	*Euplotes aediculatus* *Euplotes vannus* *Euplotes octocarinatus*	**cys-75** (ile/asp); **ile-136** (leu) **gly-137** (val); **his-139** (asn) **ser-347** (cyst)
Subclass Oxytrichia (Euhypotrichs)	*Oxytrichia granulifera* *Stylonychia lemnae* *Histriculus cavicola*	**gln-128** (asn) **leu-317** (met)
Class Oligohymenophorea	*Tetrahymena pyriformis* *Tetrahymena thermophila* *Frontonia* sp.	**asn-300** (ser) **ser-316** (cys)

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