BioOne COMPLETE

Phylogenetic Relationships between Vorticella convallaria and Other Species Inferred from Small Subunit rRNA Gene Sequences

Authors: Itabashi, Takeshi, Mikami, Kazuyuki, Fang, Jie, and Asai, Hiroshi

Source: Zoological Science, 19(8) : 931-937

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.19.931

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://complete.bioone.org/journals/Zoological-Science on 16 Aug 2022
Terms of Use: https://complete.bioone.org/terms-of-use

ZOOLOGICAL SCIENCE **19**: 931–937 (2002) © 2002 Zoological Society of Japan

Phylogenetic Relationships between *Vorticella convallaria* and Other Species Inferred from Small Subunit rRNA Gene Sequences

Takeshi Itabashi[1]*, Kazuyuki Mikami[2], Jie Fang[1] and Hiroshi Asai[1]

[1] *Department of Physics, School of Science and Engineering, Waseda University, Okubo, Shinjuku-ku, Tokyo 169-0072, Japan*
[2] *Environmental Education Center, Miyagi University of Education, Aramaki-aza-Aoba, Sendai, 980-0845, Japan*

ABSTRACT—Vorticellid ciliates generally dwell in freshwater. In nature, the species have up until now been identified by comparison with previous descriptions. It is difficult to identify between species of the genus *Vorticella*, because the morphological markers of vorticellid ciliates described in reports are limited and variable. Unfortunately, culturing them has only succeeded with certain species such as *Vorticella convallaria*, but many others have been impossible to culture. To find out whether the sequence of a small subunit rRNA gene was an appropriate marker to identify vorticellid ciliates, the gene was aligned and compared. Finding a new convenient method will contribute to research on vorticellid ciliates. In strains of *V. convallaria*, classified morphologically, some varieties of the SSrRNA gene sequences were recognized, but there were large variations within the same species. According to the phylogenetic tree, these strains are closely related. However, the difference was not as big as between *Vorticella* and *Carchesium*. In addition, *Carchesium* constructed a distinct clade from the genus *Vorticella* and *Epistylis*. These results show the possibility that the SSrRNA gene is one of the important markers to identify species of *Vorticella*. This study is first to approach and clarify the complicated taxa in the genus *Vorticella*.

Keyword: *Vorticella, Carchesium*, small subunit rRNA, identification, Peritrichia

INTRODUCTION

The vorticellid ciliate, *Vorticella*, adheres to the surface of materials such as water plants with stalks. Anyone who has had experience with the identification of free-living ciliates will understand the difficulty of distinguishing between species of *Vorticella*. Their various body shapes, variable size and highly contractile nature makes them among the most difficult of all ciliates to study and identify. This has resulted in the creation of numerous species and varieties, many of which are of doubtful taxonomic status.

With so many taxa, the descriptions of which can be found in other reports, the task of the ecologist in trying to identify individual isolates has become particularly onerous, even with the aid of identification keys such as those of Stokes (1885), Kahl (1935), Green (1974) and Warren (1986). Warren (1986) provided drawings and descriptions of the extant 82 species of *Vorticella* when he compiled the taxonomic status of the many species. But these 82 species did not include all *Vorticella* species. We have tried to identify species and constantly have had to refer to previous studies.

It is important that research at the molecular level should be based on a consistent species of organisms. One reason identification is quite difficult is because the morphological features are too vague. *Vorticella* have to be collected from a natural source because their culture is also difficult. Each classification of *Vorticella* is typically based on morphological features such as cell size and shape, cytostome, nuclear characteristics and cortical patterns. However, the morphological characteristics of *V. convallaria* are not distinguishable when the ambiguous form is found under certain culture conditions and among strains collected from nature. Many forms of *V. convallaria* from nature have been reported (Sladecek, 1971). Ciliate literature is confused with varieties of ciliates from *V. convallaria*. According to other reports, the two species used for this research were classified into the *V. convallaria* based on zooid size, inverted bell shape, situation of contractile vacuole, J-shaped macronuclear and characteristic stalk contraction (Warren, 1986).

* Corresponding author: Tel. +81-3-5286-3438;
FAX. +81-3-3200-2567.
E-mail: 601q5011@mn.waseda.ac.jp

Downloaded From: https://complete.bioone.org/journals/Zoological-Science on 16 Aug 2022
Terms of Use: https://complete.bioone.org/terms-of-use

As for *Vorticella*, mass cultivation has been reported (Vacchiano, 1991). However, long-term culture of the species collected from nature has not been possible. In most cases of molecular works, experiments should be with short-term cultures collected from nature. Therefore, we faced the difficulty whether sometimes the species was exactly the same as those reported in other works. Thus correct identification is important.

The purpose of this research was to evaluate the effectiveness of comparing partial SSrRNA gene sequencing to identify species of the genus *Vorticella*. The phylogeny of vorticellid ciliates is also discussed.

MATERIALS AND METHODS

Source of species and culturing

Stock cultures of the strains *Vorticella convallaria* Chicago, *V. convallaria* Tokyo and *Vorticella* sp. Tianjin were kept in our laboratory. Each cell of *Carchesium polypinum* Miyagi and *Epistylis* sp. Miyagi were isolated from water at a sewerage works and grown to be clones (Miyagi, Japan). The cells were cultured at 25°C in a solution mixed with natural mineral water (Volvic, France) and a lettuce medium that was inoculated with *Klebsiella pneumoniae* (Hiwatashi, 1968). Live cells were observed with Nomarski interference contrast.

It was difficult to collect a lot of cells efficiently because vorticellid ciliates are known to adhere to the bottom of glass vessels. Especially in the genus *Vorticella*, where individual cells grow at a distance from each other. Trying to collect many adhering cells from glass vessels was not an efficient method, so in the present research we mainly used the swimming form, “telotroch”, because it was easier to collect.

DNA extraction and sequencing

Swimming form cells were collected by centrifugation. About $4{\times}10^3$ cells were collected from 50ml of culture medium and washed once in distilled water. After centrifugation, the pellet was transferred into an NDS solution (1% SDS, 50mmol/L Na_EDTA, 100mmol/L Tris-HCl pH9.5) and inoculated for 10 minutes at 65°C. Finally, DNA was extracted with phenol-chloroform and precipitated with ethanol.

The rRNA genes were amplified by a PCR method using the forward primer 60f (5'-CRGYGAAACTGCTGCGAATGGCTC-3') and the reverse primer 1,160r (5'-CCMGTGTTGAGTCAAATT-AAGCCGC-3'). The forward and reverse primers were created at 60bp and 1,160bp, respectively, from the 5'-ends of the SSrRNA gene sequence in *V. convallaria* (AF070700). PCR products were isolated by the electrophoresis and purified with Geneclean III Kit (Bio 101 CA, USA). The purified DNA was sequenced directly on an ABI PRISM 310 Genetic Analyzer (PE Biosystems, CA, U.S.A.).

Sequence analysis

The accession Nos. in GenBank/EMBL/DDBJ of the sequences of the nucleic acids used for this paper were: *Epistylis plicatilis* (AF335517), *E. urceolata* (AF335516), *E. chrysemydis* (AF335514), *E. hentscheli* (AF335513), *E. wenrichi* (AF335515), *Opisthonecta henneguyi* (X56531), *Paramecium caudatum* (AF217655), *Tetrahymena thermophila* (M10932), *Vorticella campanula* (AF335518), *V. convallaria* (AF070700), *V. microstoma* (AF070701).

Sequence alignment was performed with CLUSTAL W through the WWW server of DNA Data Bank of Japan (DDBJ; Mishima, Japan). Similarity degrees between vorticellid ciliates were calculated using GeneDoc ver2.6.001. PUZZLE ver. 4.0.2 (Maximum likelihood method: Strimmer and von Haeseler, 1999) was used to construct maximum-likelihood trees with quartet puzzling support value for the internal branches and maximum likelihood branch lengths. Phylogenetic tree constructions were drawn using the program Tree View (Page, 1996).

RESULTS

Two strains of *Vorticella convallaria* for this research have different morphotypes (Fig.1A, B.). *V. convallaria* strain Chicago differs from strain Tokyo in cell length and the shape beneath the peristomial lip. Strain Tokyo was constricted beneath the peristomial lip more than strain Chicago. The cell body of *V.* sp. conformed to most of the characteristics of *V. convallaria*, except for the pattern of spasmoneme contraction. *V.* sp. had an awkward style of contraction. As a result, *V.* sp. strain Tianjin couldn't be classified into *V. convallaria*.

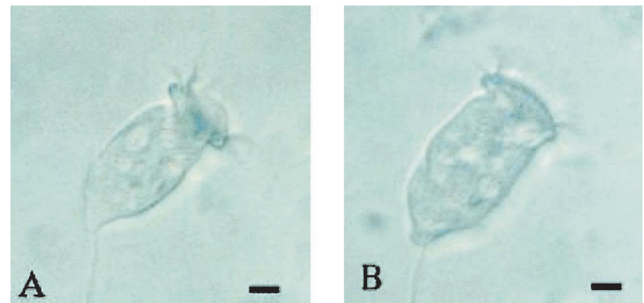

Fig. 1. Morphological variability of *Vorticella convallaria*. (A) trophont of *V. convallaria* strain Tokyo. (B) trophont of *V. convallaria* strain Chicago. Bar=10 μm.

Amplification of vorticellid genomic DNA with primers complementary to the specific parts of eukaryotic SSrRNA coding regions yielded a product about 1,100 nucleotides long. Accession Nos. of EMBL/GenBank/DDBJ and the length of partial SSrRNA gene sequenced in this experiment were as follows: *V. convallaria* strain Chicago 1,001 nucleotides (AB074082); *V. convallaria* strain Tokyo 991 nucleotides (AB074081); *V.* sp. strain Tianjin 1,003 nucleotides (AB074083); *Carchesium polypinum* strain Miyagi 962 nucleotides (AB074079); *Epistylis* sp. strain Miyagi 986 nucleotides (AB074080). The sequences of *Vorticella* species were aligned with *V. convallaria* (AF070700), *V. campanula* (AF335518) and *V. microstoma* (AF070701), and compared (Fig.2).

The genus *Vorticella* has a low G+C content in partially fixed SSrRNA gene sequences (Chicago 43%; Tokyo 42%; Tianjin 43%), similar to that found in *Carchesium* and *Epistylis* (*Carchesium* 45%; *Epistylis* 42%). In the genus *Vorticella*, the SSrRNA gene sequences had differences among species. The sequences of the *Vorticella* SSrRNA gene had many variations near 5'-ends, while the sequences of *Carchesium* SSrRNA gene, especially at the middle position, had fixed sequences. There was a specific region for only *V.* sp. near 410-bp from 5'-ends(14-bp). Differences in

Downloaded From: https://complete.bioone.org/journals/Zoological-Science on 16 Aug 2022
Terms of Use: https://complete.bioone.org/terms-of-use

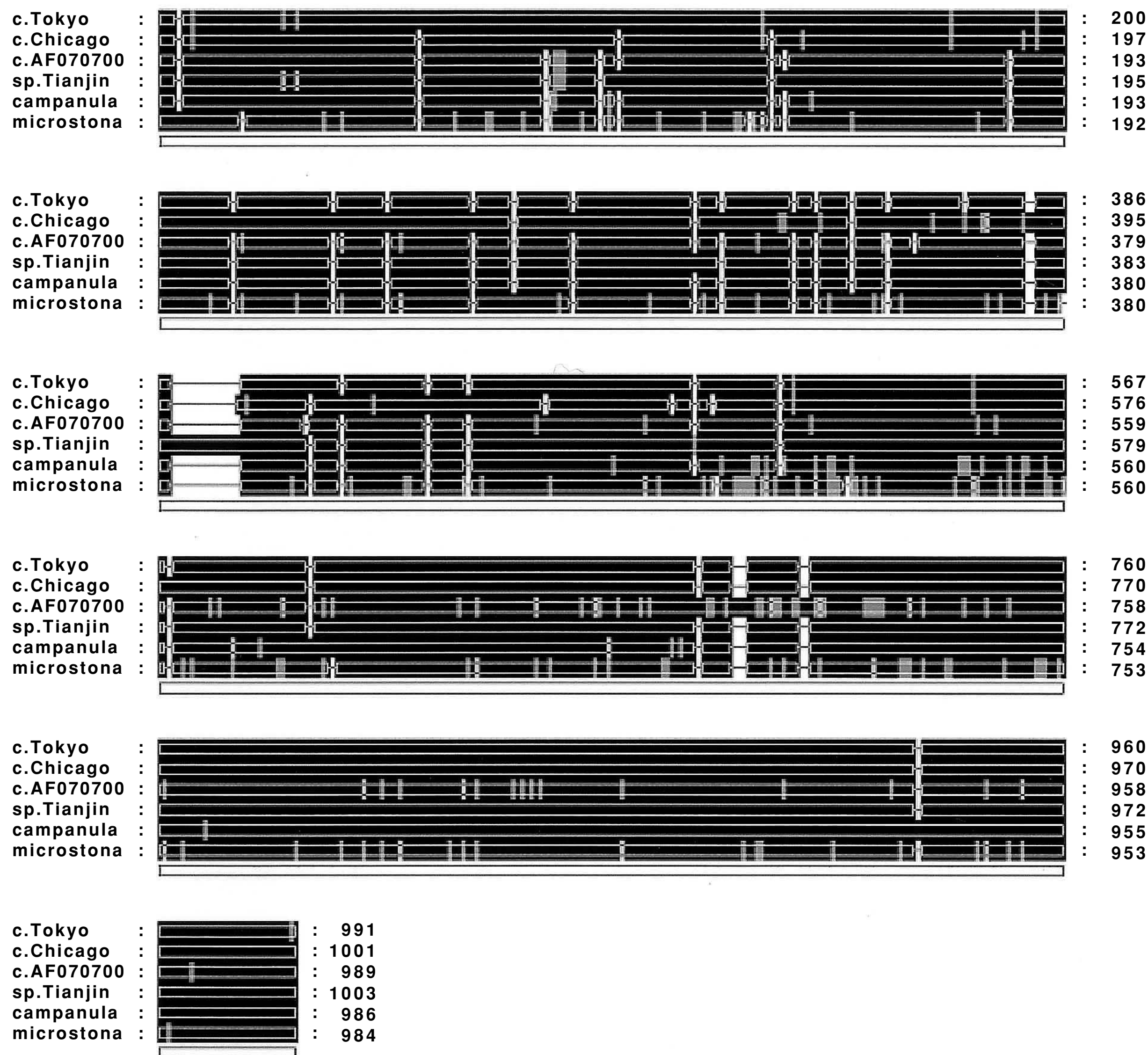

Fig. 2. Alignment of SSrRNA sequences of *Vorticella convallaria* and other species. The blank shows deletions or insertions, and bases of a different color are substituted. [c.Tokyo] *Vorticella convallaria* strain Tokyo; [c. Chicago] *Vorticella convallaria* strain Chicago; [AF070700] *Vorticella convallaria* AF070700; [ap. Tianjin] *Vorticella* sp. strain Tianjin; [microstoma] *Vorticella microstoma* AF070701; [campanula] *Vorticella campanula* AF3355183

Table 1. Similarity values for vorticellid SSrRNA sequences. The upper triangle shows the rate of similarity. The lower triangle shows the rate of deletions or insertions for all pairs.

Species/Strain	1	2	3	4	5	6	7	8
1. *V. convallaria* (Tokyo)	–	96%	90%	96%	95%	85%	76%	88%
2. *V. convallaria* (Chicago)	2%	–	88%	94%	92%	83%	76%	86%
3. *V. convallaria* (AF070700)	1%	3%	–	90%	89%	82%	71%	83%
4. *V.* sp. (Tianjin)	2%	3%	2%	–	94%	84%	75%	88%
5. *V. campanula*	1%	2%	1%	2%	–	85%	77%	88%
6. *V. microstoma*	2%	3%	2%	2%	1%	–	70%	83%
7. *C. polypinum* (Miyagi)	6%	6%	6%	6%	5%	6%	–	75%
8. *E.* sp. (Miyagi)	1%	3%	1%	2%	1%	2%	5%	–

Downloaded From: https://complete.bioone.org/journals/Zoological-Science on 16 Aug 2022
Terms of Use: https://complete.bioone.org/terms-of-use

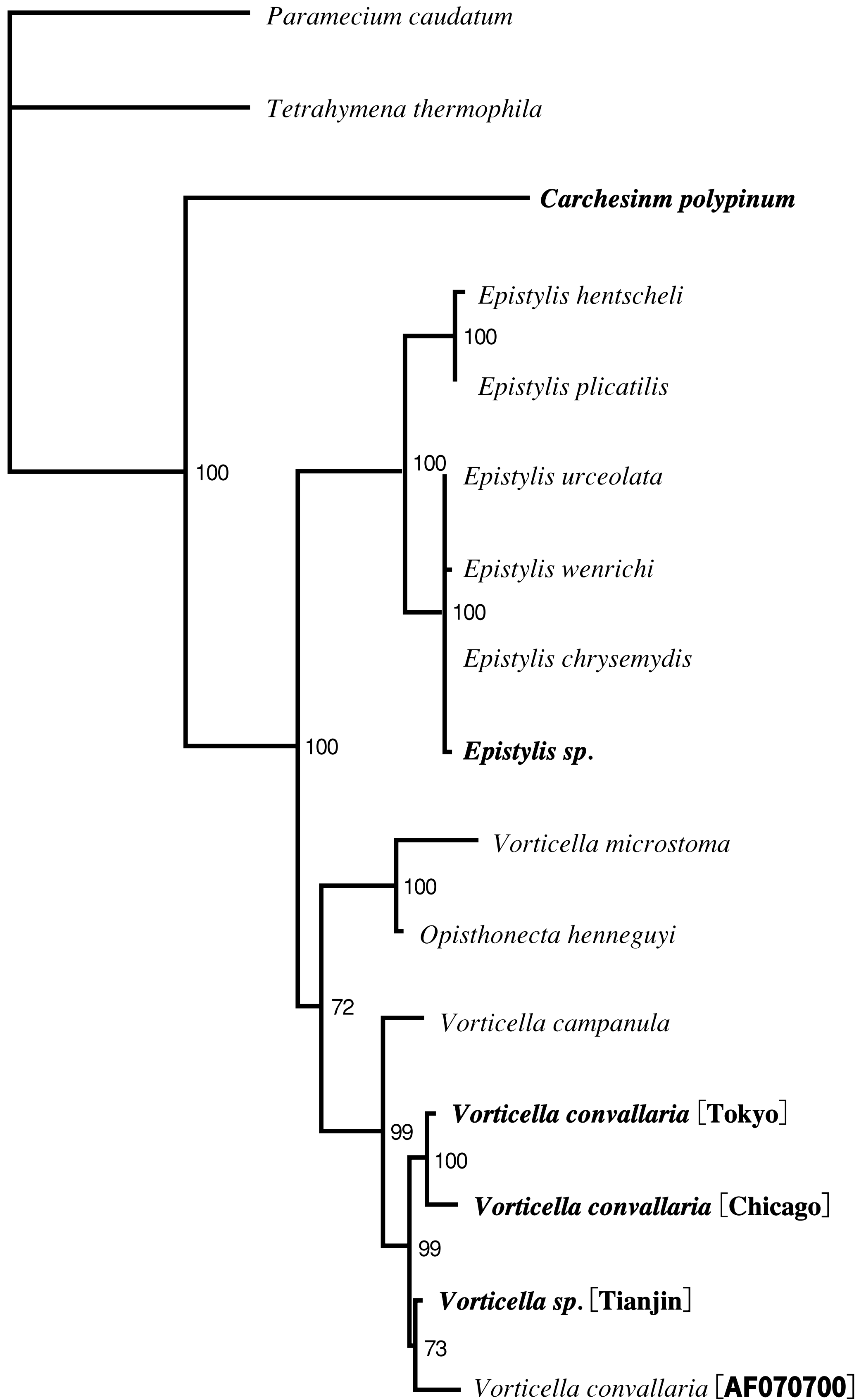

Fig. 3. A maximum likelihood tree inferred from the SSrRNA gene sequences. The numbers are quartet puzzling support values for the internal branch, while the branch lengths reflect maximum-likelihood data. Species in bold are new sequences for this study.

Downloaded From: https://complete.bioone.org/journals/Zoological-Science on 16 Aug 2022
Terms of Use: https://complete.bioone.org/terms-of-use

the sequences came out noticeably among the three strains of *V. convallaria*. Comparisons of the SSrRNA gene sequences were made from some clones of each vorticellid ciliates: no differences were found (data not shown).

Similarity values for vorticellid SSrRNA sequences showed apparent differences in the same species (Table 1). For example, the maximum and minimum similarity values were respectively 96% between the strain Tokyo and the strain Chicago, and 88% between the strain Chicago and AF070700. There were many variations of SSrRNA gene sequences in the same species. Similarity values between all of the *V. convallaria* strains and *V. microstoma* strain was from 82% to 85%. *V. campanula* showed a higher similarity to *V. convallaria* species rather than *V. microstoma*. *Carchesium* had more deletions or insertions than other vorticellid ciliates (the gap per cent; 5–6%). Furthermore there was little similarity between *Carchesium* and other vorticellid ciliates (70–76%). *Epistylis* had a higher similarity to the genus *Vorticella* rather than *Carchesium* (83–88%).

The SSrRNA sequences of vorticellid and other ciliates aligned with gaps were 1065 nucleotides and were used to generate a phylogenetic tree. These sequences corresponded to positions 121–1109 of the *V. convallaria* (AF070700) SSrRNA gene. The maximum-likelihood analysis and the constructed tree showed inferred phylogenetic relationships of vorticellid ciliates. *Paramecium tetrauleria* was chosen as the out-group species. The species that belongs to Peritrichia formed one cluster (Fig.3). Three different strains of *V. convallaria*, *V. campanula* and *V*. sp. formed a strongly monophyletic group (73–100%). However *V. microstoma* were grouped with *Opisthonecta henneguyi* rather than the other *Vorticella* species. In this study, *Carchesium polypinum* separated and revealed a considerable distance from the other vorticellid ciliate clusters.

DISCUSSION

For a long time, the taxonomy of the genus *Vorticella* has been based on macro-morphological descriptions such as the shape and size of the cell, cilia, and the form of the macronuclei. It has been difficult to choose a morphological character as a phylogenetic marker, the macro-morphological information being insufficient to identify the species, especially in the genus *Vorticella*. The vorticellid ciliates inhabit various environments, but culture has been restricted to a few strains of vorticellid ciliates. According to our experience, most of the cells collected from nature were initially able to grow but eventually stopped when they grew into a certain quantity. Therefore, we had to repeat the isolation from nature for some experiments. If the gene sequence data, like the small subunit rRNA gene sequences, were effective for identification of *Vorticella* species, it would be possible to study them using collected cells without misidentifying the species.

The taxonomy of a subclass in the Oligohymenophorea class has been established by previous research. For example, the phylogenetic relationships within the genus *Paramecium* were confirmed by molecular data of SSrRNA gene sequences (Struder-Kypke, 2000a, b). The phylogenies among the genus *Tetrahymena* were also defined by inferring from SSrRNA gene sequences and histone H4 proteins (Brunck, 1990; Sadler, 1992; Wright, 1995). But information about the gene of the vorticellid ciliates has been limited, as against many extant species. Additionally SSrRNA gene sequences of the genus *Vorticella* are restricted to three species though there are descriptions of many species in previous studies (*Vorticella convallaria*, *V. microstoma* and *V. campanula*). Therefore, additional species within Peritrichia are needed to give a strong confirmation of the relationships of the class Oligohymenophorea (Greenwood, 1991). Miao *et al*. (2001) showed that within the subclass Peritrichia, species were separated into two clades: one including colonial *Epistylis*, and the other solitary *Vorticella* and *O. henneguyi*. Both *Carchesium* and *Vorticella* have been classified into the same family Vorticellidae morphologically (Ehrenberg, 1838). *Carchesium* was separated from the genus *Vorticella* in the phylogenetic tree (Fig.3). The present result suggests the possibility that clarifying a relationship between *Carchesium* and *Vorticella* is more remote than the prospect of morphological classification. Our results suggested that the subclass Peritrichia were separated into three clades according to the phylogenetic tree based on partial SSrRNA gene sequences.

Vorticellid ciliates and the genus *Tetrahymena* belong to the Oligohymenophorea class. *Tetrahymena* has been considered to be a genus in which morphological stability has been maintained (Nanney, 1998), while there has been considerable variation in molecular constituents of the cells (Borden, 1977; Williams, 1986). However, a few differences in the sequence have been found among closely related *Tetrahymena* species in small subunit rRNAs (Jerome, 1996; Sogin, 1986; Wright, 1995) and in large subunit rRNAs (Nanney, 1998). Both *Tetrahymena* and *Paramecium* can show all the mating crosses required for species identification. In this study, SSrRNA gene sequences revealed the existence of a lot of differences among the same species of *V. convallaria*. Specifically, there were more insertions and deletions in SSrRNA gene sequences of the genus *V. convallaria* than in one of the genus *Tetrahymena*. The similarity value of the genus *Vorticella* was lower than one between the genus *Tetrahymena* (over 98%; Wright, 1995). The variation existed in both SSrRNA gene sequences as well as the forms of *V. convallaria*. Accordingly, the genus *Vorticella* did not seem to maintain morphological stability. However, it was possible to identify the species on the phylogenetic tree based on SSrRNA gene sequences, because strains having the main morphological characteristic of *V. convallaria* formed the monophyletic group. In this research, the sequence regions compared may have been too short and the compared species too small to resolve relationships within the genus *Vorticella*. There is only one report about the genus *Epistylis* and no information about

Downloaded From: https://complete.bioone.org/journals/Zoological-Science on 16 Aug 2022
Terms of Use: https://complete.bioone.org/terms-of-use

the degree of the SSrRNA gene sequence variation among the different stocks in the same species of other ciliates. Relationships within the genus *Vorticella* are poorly resolved on the available sequence information.

In the cells morphologically classified into *Vorticella convallaria*, there are variations in SSrRNA gene sequences. The variation in the same species is as large as the one among the different species. The morphological cell type of *V. convallaria* is different from those of *V. campanula* and *V. microstoma*. According to the phylogenetic tree *V. convallaria* formed a monophyletic group with *V*. sp. having the morphological characteristic of *V. convallaria*. The contractile pattern of spasmoneme was not an important phylogenetic character. Our two subgroups in the genus *Vorticella* were related by the size of the peristomial lip in relation to the maximum body width; *V. convallaria* and *V. campanula* have a peristomial lip diameter equal to, or greater than, respectively, the maximum body width while the one of *V. microstoma* was distinctly less than the greatest body width. In the genus *Epistylis*, subgroups were related based on the thickness of the peristomial lip (Miao *et al.*, 2001). The peristomial area may thus be an important phylogenetic character within vorticellid ciliates.

However, our results don't support a monophyly of the genus *Vorticella*. This was also reported by Miao *et al.* (2001), that is that *V. convallaria* grouped with *O. henneguyi*, and not the other *Vorticella* species in the phylogenetic tree using SSrRNA complete sequences. Because of this, we considered, for example, the following: that the current classification was unsuitable, that identification erred, that data for analysis was unsuitable, and that the differentiation of the gene did not reflect the diversification of the taxa. If either *O. henneguyi* or *V. microstoma* were misidentified, the genus *Vorticella* formed the monophyly. Therefore, the classification of the genus *Vorticella* is more complicated than that it was thought, and. it is necessary to acquire molecular sequence information to clarify the relationships between *Vorticella* and *O. henneguyi*.

These results show the possibility that the SSrRNA gene is one of the important markers for identifying species of *Vorticella*. The analysis of the SSrRNA gene sequence may be able to be used to distinguish *V. convallaria* collected from nature, and the variations of the SSrRNA gene sequence suggest a possibility that there are a number of variations in the gene of 20kDa calcium-binding protein (Spasmin) in the same species. The identification of species that can be seen to have been based on unsuitable markers of morphological characteristics may have caused this difficulty in species identification. Morphological diversification using outdated descriptions and phylogenetic complications within the genus *Vorticella* have also helped give rise to these difficulties. The analysis of other gene information and additional species may reveal important elements of morphological characteristics as identified markers.

ACKNOWLEDGMENTS

We thank Dr. Che Ning (Tianjin University of Light Industry) for providing us with strains of *Vorticella* sp. strain Tianjin.

REFERENCES

Borden D, Miller ET, Whitt G S, and Nanney DL (1977) Electrophoretic analysis of evolutionary relationships in *Tetrahymena*. Evolution 31: 91–102

Brunk CF, RW Kahn, LA Sadler (1990) Phylogenetic relationships among *Tetrahymena* species determined using the polymerase chain reaction. J Mol Evol 30: 290–297

Ehrenberg CG (1838) Die infusionsthiere als vollkommene organismen. Verlag von Leopold Voss, Leipzig, p 548

Green J (1974) Parasites and epibionts of Cladocera. Trans Zool Soc London 32: 417–515

Greenwood SJ, Sogin ML, Lynn DH (1991) Phylogenetic relationships within the class Oligohymenophorea, phylum Ciliophora, inferred from the complete small subunit rRNA gene sequences of *Colpidium campylum, Glaucoma chattoni*, and *Opisthonecta henneguyi*. J Mol Evol 33: 163–174

Hiwatashi K (1968) Determination and inheritance of mating type in *Paramecium caudatum*. Genetics 58: 373–386

Jerome CA, Lynn DH (1996) Identifying and distinguishing sibling species in the *Tetrahymena pyriformis* complex (Ciliophora, Oligohymenophorea) using PCR/RFLP analysis of nuclear ribosomal data. J Euk Microbiol 43: 492–497

Kahl A (1935) Urtiere der Protozoa. I: Wimpertiere oder Ciliata (Infusoria), eine bearbeitung der freilebenden und ectommensalen Infusorien der Erde, unter Ausschluss der marinen Tintinnidae. 4. Peritricha und Chonotricha. In: "*Die Tierwelt Deutschlands*" Ed by G Grrimpe, E Wagler, Verlag von Gustav Fischer, Jena, pp 651–864

Miao W, Yu Y-H, Shen Y-F (2001) Phylogenetic relationships of the subclass Peritrichia (Oligohymenophrea, Ciliophora) with emphasis on the genus *Epistylis*, inferred from small subunit rRNA gene sequences. J Euk Microbiol 48:583–587

Nanney DL, Nyberg D, Chen SS, Meyer EB (1980) Cytogeometric constraints in *Tetrahymena* evolution: Contractile vacuole pore positions in nineteen species of the *Tetrahymena pyriformis* complex. Am Nat 115: 705–717

Nanney DL, Park C, Preparata R, Simon EM (1998) Comparison of sequence differences in a variable 23S domain among sets of cryptic species of ciliated protozoa. J Euk Microbiol 45: 91–100

Noland LE, Finley HE (1931) Studies on the taxonomy of the genus *Vorticella*. Trans Amer Microscop Soc 50: 81–123

Page RDM (1996) TreeView: an application to display phylogenetic trees on personal computers. Comp Appl Biosc 12:357–158

Sadler LA, CF Brunk (1992) Phylogenetic relationships and unusual diversity in histone H4 proteins within the *Tetrahymena pyriformis* complex. Mol Biol Evol 9: 70–84

Sladecek V (1971) Saprobic sequences within the genus *Vorticella*. Water Res 5: 1135–1140

Sogin ML, Ingold A, Karlok M, Nielsen H, Engberg HJ (1986) Phylogenetic evidence for the acquisition of ribosomal RNA introns subsequent to the divergence of some of the major *Tetrahymena* groups. EMBO J 5: 3625–3630

Stokes AC (1885) Key to vorticellae. Microscope 5: 97–101

Strimmer K, von Haeseler A (1999) Puzzle-maximum likelihood analysis for nucleotide, amino acid, and two-state data. Version 4.0.2

Struder-Kypke MC, Wright A-D G, Forkin SI, Lynn DH (2000a) Phylogenetic relationships of the genus Paramecium inferred from small subunit rRNA gene sequences. Mol Phylogenet Evol 14: 122–130

Downloaded From: https://complete.bioone.org/journals/Zoological-Science on 16 Aug 2022
Terms of Use: https://complete.bioone.org/terms-of-use

Struder-Kypke MC, Wright A-D G, Forkin SI, Lynn DH (2000b) Phylogenetic relationships of the subclass peniculia (Oligohymenophorea, Ciliophora) Inferred from Small Subunit rRNA Gene Sequences. J Euk Microbiol 47: 419–429

Vacchiano EJ, Kut JL, Wyatt ML, Buhse Jr. HE (1991) A novel method for mass-culturing *Vorticella*. J Protozool 38: 608–613

Warren A (1986) A revision of the genus *Vorticella* (Ciliophora: Peritrichida). Bull Br Mus nat Hist (Zool) 50:1–57

Williams NE (1986) Evolutionary change in cytoskeletal proteins and cell architecture in lower eukaryotes. Prog Protistol 1: 309–324

Wright A-D G, Lynn DH (1995) Phylogeny of the fish parasite *Ichthyophthirius* and its relatives *Ophryoglena* and *Tetrahymena* (Ciliophora, Hymenostomatia) inferred from 18S ribosomal RNA sequences. Mol Biol Evol 12: 285–290

(Received November 20, 2001 / Accepted May 14, 2002)

Downloaded From: https://complete.bioone.org/journals/Zoological-Science on 16 Aug 2022
Terms of Use: https://complete.bioone.org/terms-of-use