

Description of *Campanella hanchuanensis* p. nov. (Ciliophora, Peritrichida): General Morphology and Isozyme Pattern

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ABSTRACT

Living and stained specimens of *Campanella hanchuanensis* sp. nov., collected near Hanchuan Power Plant, Hubei Province, China, were studied under light microscopy. Infraciliature was stained using a protargol staining technique. There is no bridge between concentric silver lines. The aboral ciliary wreath consists of 6 lines of basal bodies, with the middle 4 lines compacted and one lateral line each side separated. The other characters are similar to or the same as that described in *Campanella umbellaria*.

The esterase isozyme was analyzed by micro-isoelectrofocussing. *Campanella hanchuanensis* sp. nov. has three bands of esterase near the cathode.

INTRODUCTION

The genus *Campanella* is a cosmopolitan but small group of aloricate peritrich^[10], and up to now, only four species have been described^[1,2,5,7,10,1]. Of these species, *Campanella umbellaria* has been well studied and described using techniques of silver impregnation and transmission electron microscopy^[3,6].

In the present study, we describe the morphology and infraciliature of *Campanella hanchuanensis* sp. nov. using a light microscopy and protargol stain. This species is very different from the other 3 species of *Campanella* except for *Campanella umbellaria*. However, there are major differences between our observations and those made by former authors in *Campanella umbellaria*. The most remarkable differences pertain to the stalk shape, paroral structure, pellicle patterns and aboral ciliary wreath.

MATERIALS AND METHODS

Campanella hanchuanensis sp. nov. were isolated in May 1992 from macrophyte (*Jussiaea repens*) collected from a shallow village pond situated near Hanchuan Power Plant, Hubei Province, when the water temperature was 20°C and the pH, 6.0. Observations were made of both living organisms and protargol-stained specimens. Studies of the infraciliature were carried out using the protargol-staining method of Shi^[8]. Cell measurements were made using a calibrated ocular micrometer. Esterase isozyme was analyzed by micro-isoelectrofocussing^[12]. As soon as samples were brought to the laboratory, *Campanella hanchuanensis* sp. nov. were isolated and washed three times in 10 mM Tris-HCl buffer, pH 7.4. The pellets were homogenized in an ice bath for 2 min. After centrifugation (48,000g x 30min at 4°C), the supernatants were aliquoted and stored at -20°C. Staining method followed Simon^[9].

RESULTS

GENERAL MORPHOLOGY

Campanella hanchuanensis sp. nov. is an inverted bell-shaped ciliate (Fig.1). Measurements of living organisms are: 140-165 μm long and 100-131 μm wide. Table 1 shows the biometrical characterization of this species. Peristomal disc, not elevated, bears several turns of cilia and sometimes 4 turns can be distinguished (Fig.2). The middle part of the body is of the widest and after that, the body is drastically tapering. The posterior part always takes oblique position. That is, its axis is not that of the front (Fig.1). The body is full of granule and endoplasm is yellowish (Fig. 1). One prominent contractile vacuole is located apically, Macronucleus is C-shaped, horizontally located (Fig.3).

Stalk of *Campanella hanchuanensis* sp. nov., about 15 μm in diameter, isn't straight. Colony is dichotomously branched (Fig. 1), with the number of dichotomy being about 5, so the zooids of one colony amount to about 28.

INFRACILIATURE

From the peristomal disc to the aboral ciliary wreath, there are about 45-48 concentric silver lines and from the wreath to scopula, 26-28 and between concentric lines no bridge exist (Fig.4). The aboral ciliary wreath consists of about 6 lines of basal bodies, with the middle 4 lines compacted and one lateral line each side separated (Fig.5). Buccal ciliary apparatus is very developed. Haplokinety (H) and polykinety (PO) have 4 turns in their outer course (Fig.3,7,8). The H consists of two lines, whether inside or outside the infundibulum. In someplace, the two lines are closed and in the other site, they are separated a lot (Fig.8,9,10). The PO changes below the mouth of the infundibulum into two triple rows. The third triplet of kineties at the end of infundibulum is encircled with the cytostome in horse-shoe form (Fig.8).

ISOZYME CHARACTERIZATION

Three bands appeared in the esterase isozyme analysis. They are near the cathode (Fig.11).

DISCUSSION

Campanella is a small genus and 4 species are recorded^[5,10], namely, *C. umbellaria*, *C. tincta*, *C. purneri* and *C. liebermanni*. In the present study, a new species of *Campanella hanchuanensis* is reported. It greatly differs from *C.purneri* and *C.liebermanni* in that *C. purneri* has thicker stalks and different dichotomy and *C.liebermanni* has different shape of the body and the stalk^[4] (Fig.1). The number of turns of oral cilia distinguishes *C. hanchuanensis* sp. nov. and *C. tincta*. *C. hanchuanensis* sp. nov. has only 4 turns of cilia but *C. tincta*, 6^[6] (Fig.3,6,7). Now it turns to comparison of *C. hanchuanensis* sp.nov. and *C. umbellaria*, of which a lot of investigation was made and variation was reported in some characters.

Schroder^[6] described *C. umbellaria* well and from then on, Kahl^[2], Lom^[3] and Stiller^[11] have carried out further investigation of morphology of the species. On the whole, the morphologies described by the authors above are similar. However differences are recognized in some characters. In the more conservative infraciliature (weak character in Foissner's paper), Kahl^[2] described 4 turns of oral cilia, Schroder^[7] showed 4 and 1/2 turns and evermore, Stiller^[11] placed one species of *Campanella* with 5 turns in the same taxon. In the present study, we have described the morphology and infraciliature of the specimens we collected from Hanchuan Power Plant, Hubei Province with light microscopy and protargol stain. The specimen and *C. umbellaria* described previously are obviously similar, but remarkable difference exists. Schroder^[7] and Stiller^[10] gave the description of quadrilateral silver lines but

we didn't notice the bridge between concentric silver lines (Fig.4). Stiller^[10] gave a sketch of aboral ciliary wreath, which consists of two lines of kinetosome. It differs from the results of our observation, which have shown the presence 6 lines of kinetosome, with the middle 4 lines compacted and one lateral line each side separated (Fig.5). The infraciliature is a conservative structure and a lot of protozoologists suggests that this subcellular structure should be useful in phylogenetic analysis^[4]. The ciliate cortex is divided into two major functional regions, the somatic region and the oral region, both cortical regions are constrained by the phenomenon of structural conservatism; that is, the conservation of structure through time is inversely related to the level of biological organization. Furthermore, the evolutionary rate of change of oral features is greater than that of somatic features, probably due to developmental and ecological factors^[4]. Therefore, the specimen collected from Hanchuan Power Plant should be a new species and named *Campanella hanchuanensis* sp.nov.

Isozyme analysis has been widely used in identification of ciliate population^[8]. In the present study, we applied micro-isoelectrofocussing to analyze esterase isozyme of this specimen. It has three bands (Fig.11). The authors wish to introduce the concept of "chemical identity" into wild ciliate identification.

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Table 1. Biometrical characterization of living *C. umbellaria*

| Character (uM) | Minimum | Maximum | Range | Median | Variance | Standard deviation | Standard error | Sample size |
|----------------|---------|---------|-------|--------|----------|--------------------|----------------|-------------|
| Length | 142 | 165 | 23 | 153 | 42 | 6.5 | 1.7 | 15 |
| Width | 100 | 131 | 31 | 104 | 123 | 11.1 | 2.9 | 15 |

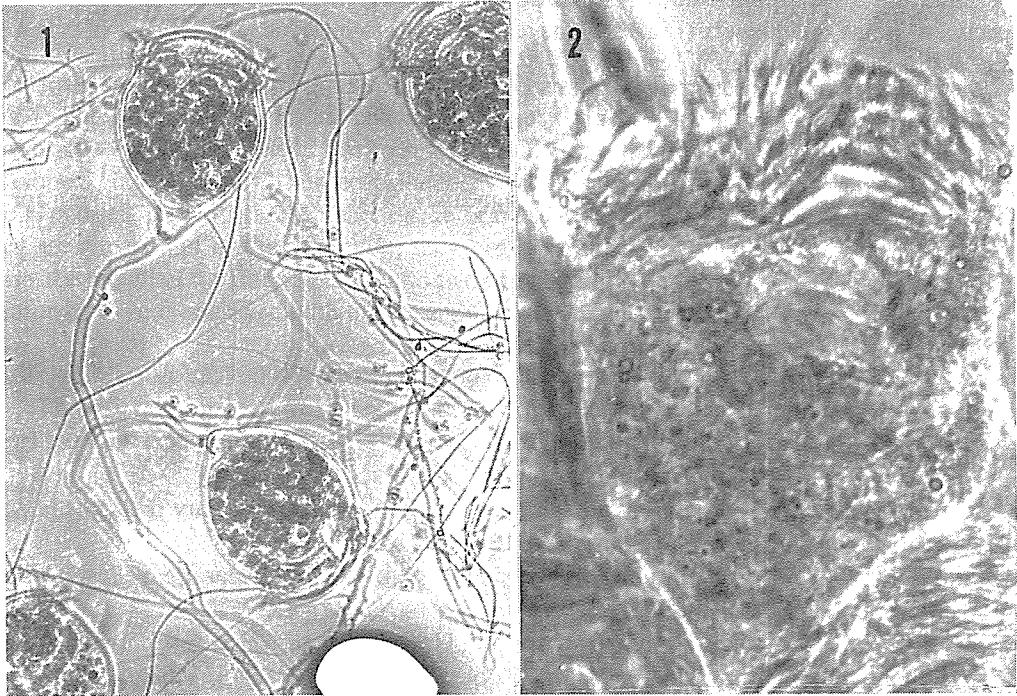


Fig.1. Small portion of living colony of *Campanella hanchuanensis* sp. nov., Noting the convoluted stalks. phase contrast(x200)

Fig.2. A living zooid of *Campanella hanchuanensis* sp. nov. showing 4 turns of oral cilia. phase contrast (x600)

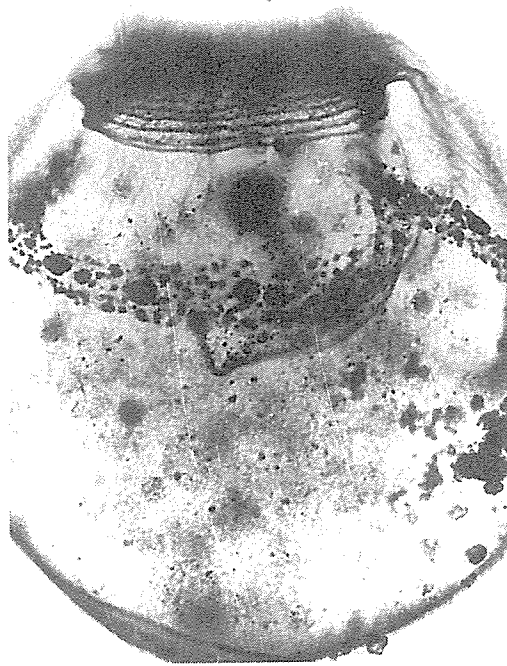


Fig.3. Lateral view of an individual of Campanella hanchuanensis sp. nov., stained with protargol showing halpokinety(H) and polykinety(PO) in their outer course and macronucleus (x700)

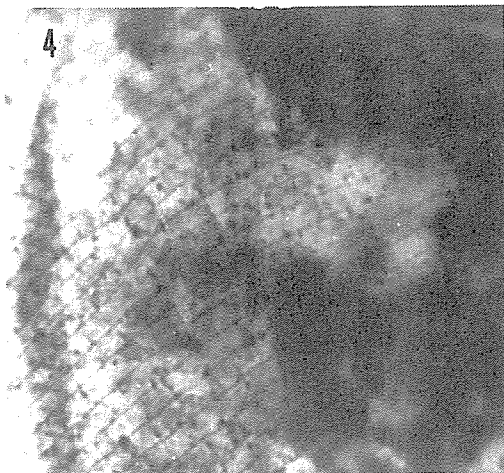


Fig.4. Lateral view of Campanella hanchuanensis sp. nov. showing the parallel concentric silver lines and no bridge between the lines (x1700)

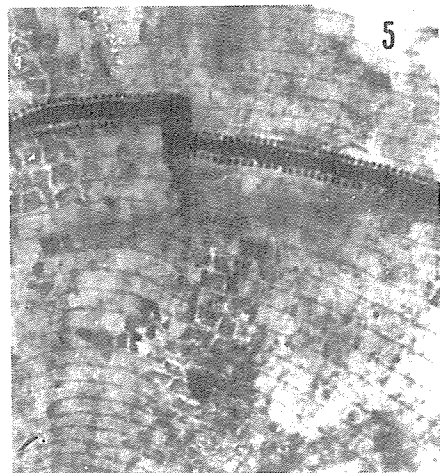


Fig.5. Lateral view of Campanella hanchuanensis sp. nov. showing aboral ciliary wreath which consists of lines of kinetosomes (x1750)

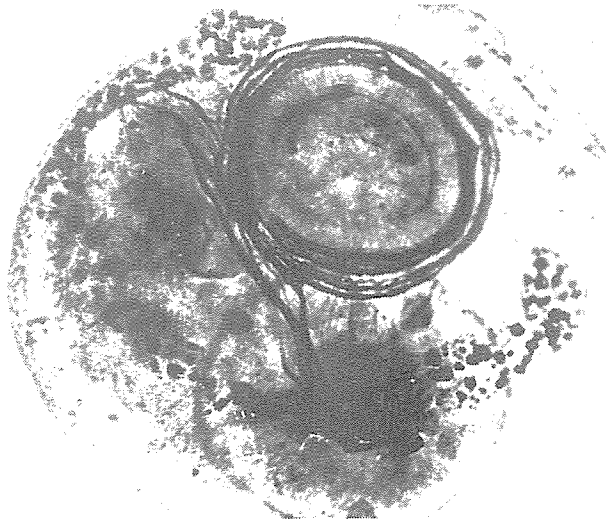


Fig.6. Apical view of Campanella hanchuanensis sp.nov. stained with protargol showing infraciliature position (x700)

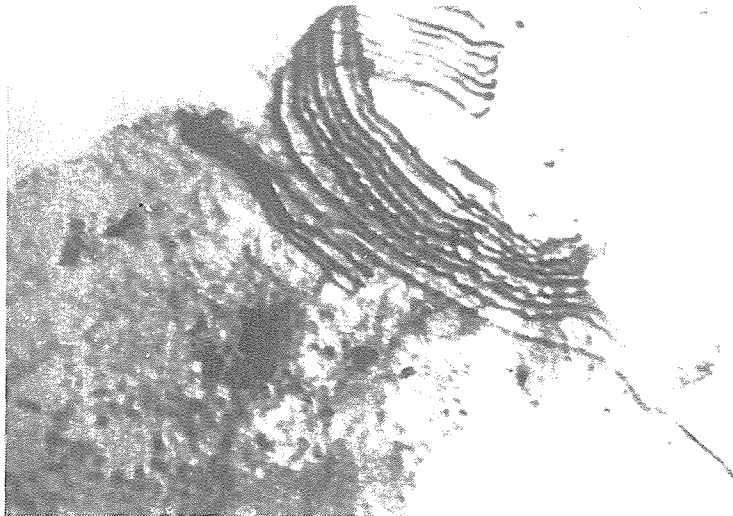


Fig.7. View of baccal infraciliature of Campanella hanchuanensis stained with protargol showing 4 turns of oral cilia(x1400)



Fig. 8. Lateral view of Campanella hanchuanensis sp. nov. stained with protargol(x700)



Fig.9. Campanella hanchuanensis sp. nov. stained with protargol showing a part of H and PO in the infundibulum (1700)

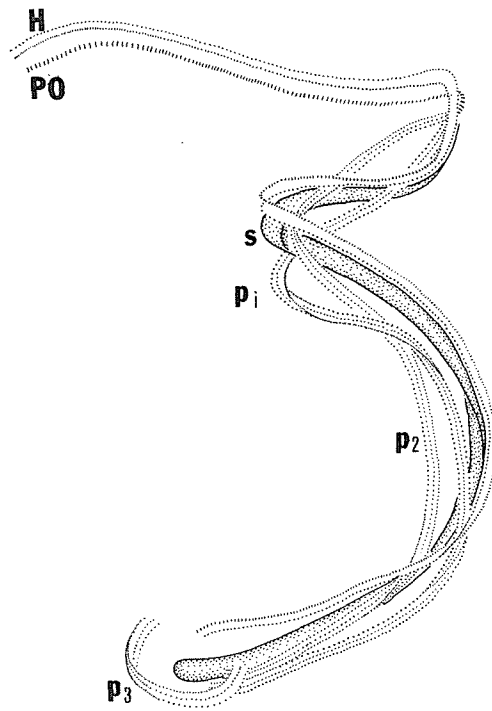


Fig. 10. *Campanella hanchuanensis* sp. nov. showing infundibular infraciliature.

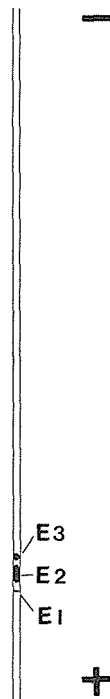


Fig. 11. Representative banding patterns of esterase isozyme for *Campanella hanchuanensis* sp. nov.