Takaaki KOBARA* & Mitsuo CHIHARA**: Spermatozoids of *Pseudodichotomosiphon constrictus* with special reference to the systematic position of the genus

高原隆明*・千原光雄**: クビレミドロの精子と属の分類上の位置について

Pseudodichotomosiphon is a monotypic marine, green, siphonous and oogamous alga and has long been a subject of dispute among phycologists with regards to its systematic position. The genus was described by Yamada (1934), with *P. constrictus* as the type species, separated from *Vaucheria*, on the basis of the following characteristics: 1) the presence of constriction in the frond, 2) the possession of a rhizoidal portion at the base, and 3) dichotomous ramification. Yamada (1934) placed it in the Siphonales of the Chlorophyceae, and this treatment was followed by many phycologists, including Fritsch (1935), Okamura (1936), Tseng (1936) and Hirose (1959), but was not followed by others such as Segawa (1956), Segawa & Kamura (1960) and Yamagishi (1964), who characterized it as a member of the Xanthophyceae. Recently, we have studied the pigment composition and fine structure of vegetative cells of this alga, and the results suggest the affinity of the genus to the Xanthophyceae (Hori, Kobara & Chihara 1979, Yokohama, Kobara & Chihara 1980). In the current system of classification of algae, the structure of the motile cell is used as one of the diagnostic criteria at the rank of division or class. With this in mind, we have undertaken a morphological study of the spermatozoids of *P. constrictus*. The present paper deals with the results obtained, together with some remarks regarding the systematic position of the genus.

Materials and methods Specimens bearing mature reproductive organs were collected at Chinen in the southern part of Okinawa Island, in March and April, 1983. They were brought to the laboratory near Chinen within an hour and kept in petri dishes containing sterilized sea water. For the observation of

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spermatozoids, both light and scanning electron microscopes were employed. The procedures for the electron microscopy were as follows. When spermatozoids began to swim within the antheridium, a filament was put in 2% OsO_4 solution for fixation. After dehydration by an ethanol series, the antheridium was cut off and opened, and spermatozoids were gently pushed out onto the sample table for SEM. The spermatozoids were then dried in the air, without using the critical point dryer system.

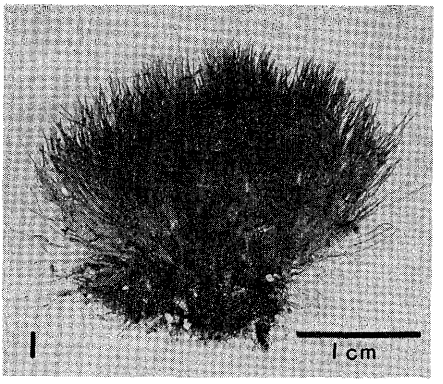
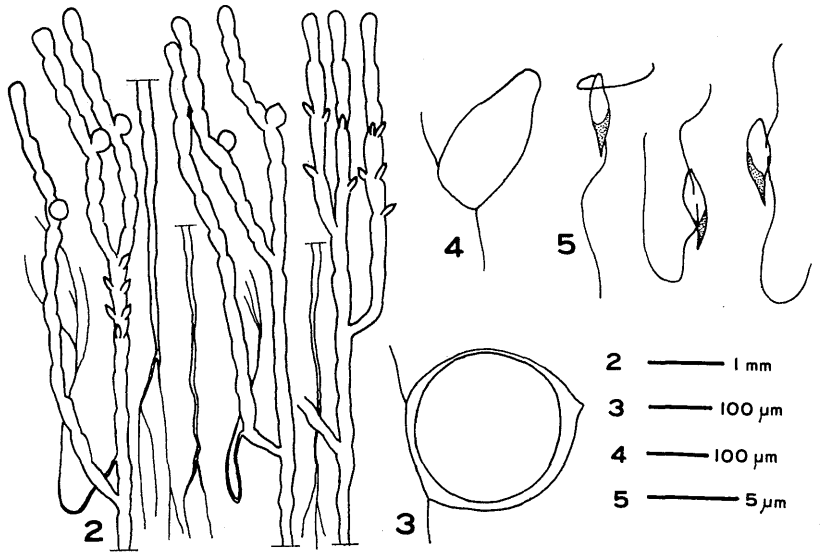


Fig. 1. Specimen of *Pseudodichotomosiphon constrictus*, collected from Okinawa Island.

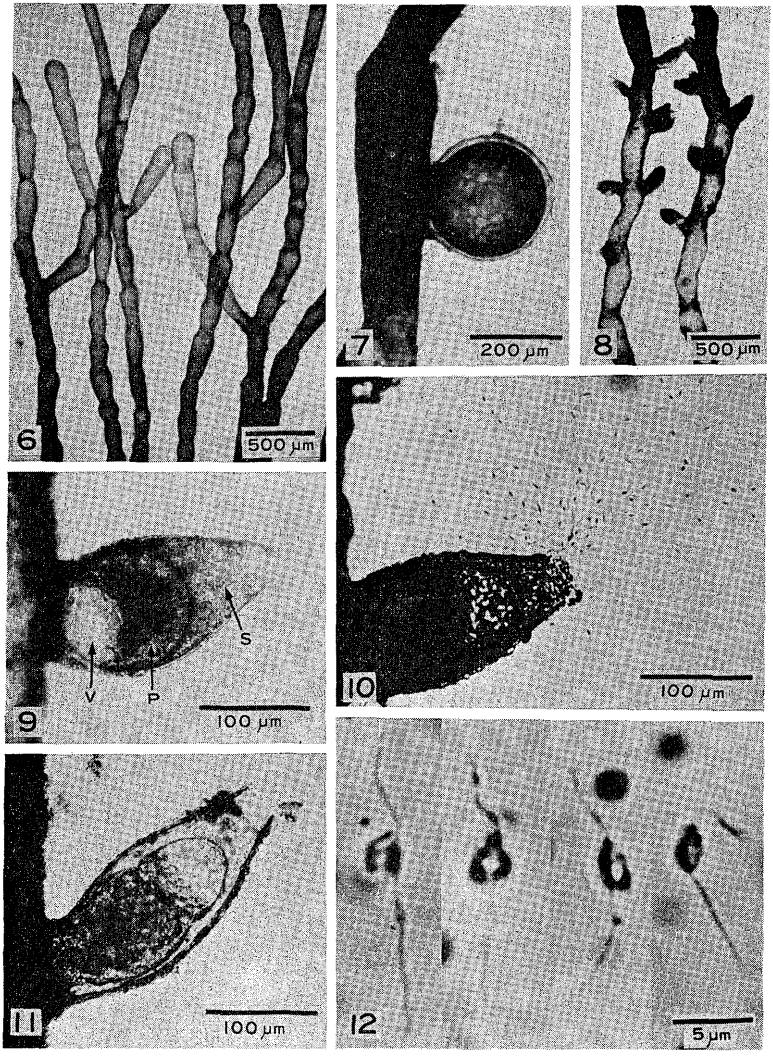
Observations and discussion The alga grew on muddy sands in the mid-intertidal zone at Chinen, Okinawa Island, forming a tuft of 1-2 cm in diameter (Fig. 1). Filaments were siphonous, appearing green, measuring 1-2 cm in height and 120-270 μm in diameter, and had constrictions (Fig. 2). They were simple or sparsely branched, with slender rhizoids at the base. The rhizoids, 20-80 μm in diameter, extended into the muddy sand and their color was reddish brown. Specimens bearing mature reproductive organs were found in spring, March to April, but not in February. Oogonia were borne laterally and usually solitarily on the filaments and were almost spheroid in shape. They lacked a stalk and measured 250-300 μm in diameter (Figs. 3, 7). They possessed a papillate appendage at the apex. The egg within the oogonium was also spheroid, measuring 220-250 μm in diameter, and contained many oil-like droplets. Antheridia were borne laterally and usually aggregately, rarely solitarily, on the filaments. They were fusiform to boat-shaped, without a stalk, and measured 170-220 μm long and 80-120 μm in width (Figs. 4, 8, 9). These morphological features agree with those of *P. constrictus* described by Yamada (1932, 1934).

During the formation of spermatozoids, the protoplasm of the antheridium was first segregated into two parts, an outer and inner part (Fig. 9). The outer part later produced numerous spermatozoids and, owing to the disintegration of chloroplasts, became whitish in color. The inner part did not undergo any cell division, but remained as it was, appearing green due to the presence



Figs. 2–5. *Pseudodichotomosiphon constrictus*. 2. Gross morphology. 3. Oogonium. 4. Antheridium. 5. Spermatozoids.

of chloroplasts. Before liberation, the spermatozoids began to move about within the antheridium, and then swam rapidly, using their flagella, and finally swam out through an opening which was formed on the conical tip of the antheridium (Figs. 10, 11). It usually took one to two minutes for their complete liberation. The inner part of the antheridium usually contained a large vacuole (Fig. 9) and, a few days after the liberation of spermatozoids, it turned to brown in color and finally disintegrated. The formation and liberation of the spermatozoids probably took place daily during the fruiting period. The spermatozoids were exceedingly small and slender, ovoidal or pear-shaped, with a tapering posterior end and measured 3–4 μm in length and about 1.5 μm in width (Figs. 5, 12). They were almost colorless, although a pale green chloroplast was located in the posterior part of the cell body. No eyespot was observed in the spermatozoids. The spermatozoids possessed two flagella laterally inserted at the ventral side: one was directed forward and the other behind. The anterior flagellum was shorter than the posterior one, the former measuring 5–7 μm in length and the latter measuring 8–11 μm in length. The liberated spermatozoids swam



Figs. 6-12. *Pseudodichotomosiphon constrictus*. 6. Erect filaments with constrictions in places. 7. Filament with oogonium. 8. Filament with antheridia. 9. Antheridium in which the content divided into outer part containing numerous spermatozoids (S) and inner protoplasm (P) containing a large vacuole (V). 10. Antheridium liberating spermatozoids from the conical tip. 11. Antheridium from which spermatozoids had been liberated. Note protoplasm remained within the antheridium. 12. Spermatozoids with two unequal flagella.

actively for a short period, showing no tendency of phototaxis, but sometimes showing rotation on their axes as they moved forward spirally. Observations with scanning electron microscope showed that the anterior flagellum possessed numerous hairly appendages on the surface, probably on both lateral sides, whereas the posterior one had no hairly appendages on its surface. The fine structure of these flagella will be described elsewhere.

From these observations, it is evident that the spermatozoids of *Pseudodichotomosiphon constrictus* are fundamentally identical with those of *Vaucheria* examined by Koch (1951) and Moestrup (1970). All of the spermatozoids of *Vaucheria* so far examined possess two laterally inserted flagella, which are unequal in length, one directed forward and the other directed backward, the former being of the tinsel-type and the latter being of the whiplash-type. When describing *Pseudodichotomosiphon* as a new genus, Yamada (1934) suggested, on the basis of morphological features, the close relationship of the genus with *Dichotomosiphon*. However, Moestrup & Hoffman (1975) have demonstrated that the flagellation of the spermatozoids of *Dichotomosiphon tuberosus* is typical of the chlorophycean algae, the flagella being two in number, of equal length and both arising at the anterior end of the cell body.

On the basis of the morphological features of the spermatozoids examined in this study and the data available at present, we suggest that *Pseudodichotomosiphon* should be place in the Xanthophyceae as an autonomous genus related with the genus *Vaucheria*.

This work was partly supported by a Grant-in-Aid for Scientific Research (no. 948077 & 534028) from the Ministry of Education, Science and Culture, Japan.

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沖縄の潮間帯の砂泥地に生育する緑色の管状藻クビレミドロ*Pseudodichotomosiphon constrictus* (Yamada) Yamada の分類上の位置については異なる二つの見解がある。一つは緑藻綱・ミル目（広義）のチョウチンミドロ科，他の一つは黄緑藻綱・フシナシミドロ目のフシナシミドロ科とするものであるが，いずれも確かな証拠がないままの意見である。われわれ（1979, 1980）はさきに栄養体の細胞構造，特に葉緑体の微細構造と光合成色素組成の研究結果から，この藻とフシナシミドロ属との近縁性を考察したが，今回，分類上の位置決定に重要な形質の一つとされる精子の放出に成功し，その形態を観察し，さきのわれわれの考察をさらに支持する知見を得ることができた。クビレミドロの有性生殖の時期は春で，生殖器官の形成は潮汐条件と関係なく毎日起るらしい。精子は細長い卵形または西洋梨形で，極めて小さく，大きさは 3-4×1.5 μm であり，側部に長短2本の鞭毛をもつ。前方にのびる鞭毛は短く，後方のそれは長い。精子がもつこれらの特徴はフシナシミドロ属のそれと基本的に同じである。

山田(1934)がフシナシミドロ属 *Vaucheria* より分離してクビレミドロ属 *Pseudodichotomosiphon* を設立した際に分類形質に用いた，藻体にくびれがある，仮根をもつ，および叉状に分枝するなどの特徴に加え，われわれがさきに指摘した葉緑体の特異な形状等を考慮すると，クビレミドロ属は分類上独立した属として扱い，フシナシミドロ科に位置させるのが妥当と思われる。