Hisayoshi NOZAKI*: **Morphology and reproduction of Japanese *Volvulina steinii* (Chlorophyta, Volvocales)****

野崎久義*: 本邦産の *Volvulina steinii* (緑藻・オオヒゲマワリ目) の形態と生殖について**

(Pl. III-IV)

The genus *Volvulina* was described by Playfair in 1915 with *V. steinii* as the type species. The validity of this genus, however, had been questioned by several phycologists (Pascher 1927) and it was established by Pocock in 1953 through a re-examination of the preserved type material and a cultural study. It is one of the less common members of the colonial Volvocales and is characterized by having 16 (rarely 8 or 4) lenticular to hemispherical cells which are embedded in individual sheaths. In addition to *V. steinii*, two species, *V. playferiana* (Skvortzow 1957) and *V. pringsheimii* (Starr 1962), have been described. However, neither species of *Volvulina* has been reported in detail from Japan. In 1980, I had a chance to collect specimens of *V. steinii* from Kanagawa Prefecture. The present paper describes the morphology and reproduction of Japanese strains of *V. steinii* observed with laboratory cultures.

Materials and Methods Soil samples used in this study were collected in a paddy field at Nagae, Hayama-cho, Kanagawa Prefecture in December 1980. Clonal cultures were obtained by the pipette-washing method (Pringsheim 1946) from petri dishes in which a small amount of the dried soil sample and a boiled pea (*Pisum sativum*) were rewetted with distilled water. The cultures were grown in screw-cap tubes (18×150 mm) containing 12 ml of modified M3-medium (Nozaki & Kasaki 1979). In addition to this synthetic medium, soil-water-pea medium (Starr 1964) was also used for observing the morphology of the vegetative phase. The cultures were kept at about 20°C, with alternating periods of 14 hr light and 10 hr dark at a light intensity of about 4000 lux provided by cool-white and pink-green, fluorescent lamps.

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Colonies of two complementary mating types were mixed in a watch glass supported on a glass triangle in petri dishes. About 5 ml of distilled water was added to the bottom of the petri dish to minimize water evaporation from the mixture. Heterothallic mating pairs of strains were obtained by random mixings of ten strains from the samples.

The method of oospore germination of *Chara* used by Sano (personal communication) was applied as follows: after the dark treatment of 2-10-day-old zygotes on the agar surface for more than three months, the zygotes were transferred from the agar surface to the new liquid medium in a double-cap tube. Nitrogen gas was substituted for the air in the double-cap tube using an injector. This double-cap tube was enclosed in red cellophane paper and placed under the usual illumination.

Gelatinous matrix and flagella were observed by staining with aniline blue or haematoxylin and/or by using a phase contrast microscope. The staining methods of Rosowski & Hoshaw (1970) were used for detecting pyrenoids.

Observations Morphology of vegetative phase. An ellipsoidal to spherical motile colony usually contained 16 cells arranged in four whorls round the periphery of the gelatinous matrix (Figs. 1, 2; Pl. III A, B). The colonies were up to 65 μm in length. The individual cells were lenticular to hemispherical in shape, measuring up to 20 μm in surface diameter. Each cell was separately embedded in an individual sheath (a keystone-shaped space) formed in the gelatinous matrix of the colony. The structure of this keystone-shaped space could be clearly observed by staining with aniline blue or haematoxylin (Pl. III C). In addition to these 16 keystone-shaped spaces, the gelatinous matrix formed a small hollow in the centre of the colony (Pl. III D).

Each cell contained a massive bowl-shaped chloroplast which was somewhat striated on the surface, and had two flagella of equal length. Two to ten or more contractile vacuoles were on the anterior surface of the cell (Fig. 1; Pl. III A). Each of the four cells in the most anterior whorl had a single stigma. In some colonies, another stigma was observed in one of the posterior cells (Fig. 2).

The chloroplast did not show pyrenoids in the younger age of the culture (Fig. 2; Pl. III A). Three days after the inoculation of the culture, however, a single pyrenoid, or sometimes two or three, appeared in each chloroplast of the cells. This pyrenoid was located in the brim of the bowl-shaped chloro-

plast (Fig. 1; Pl. III B). Using the staining methods of Rosowski & Hoshaw (1970), the pyrenoid could be clearly detected two days after the inoculation, but could not be detected in the one-day-old culture. The colonies growing in the soil-water-pea medium began to show an observable pyrenoid in each chloroplast of the cells four days after the inoculation.

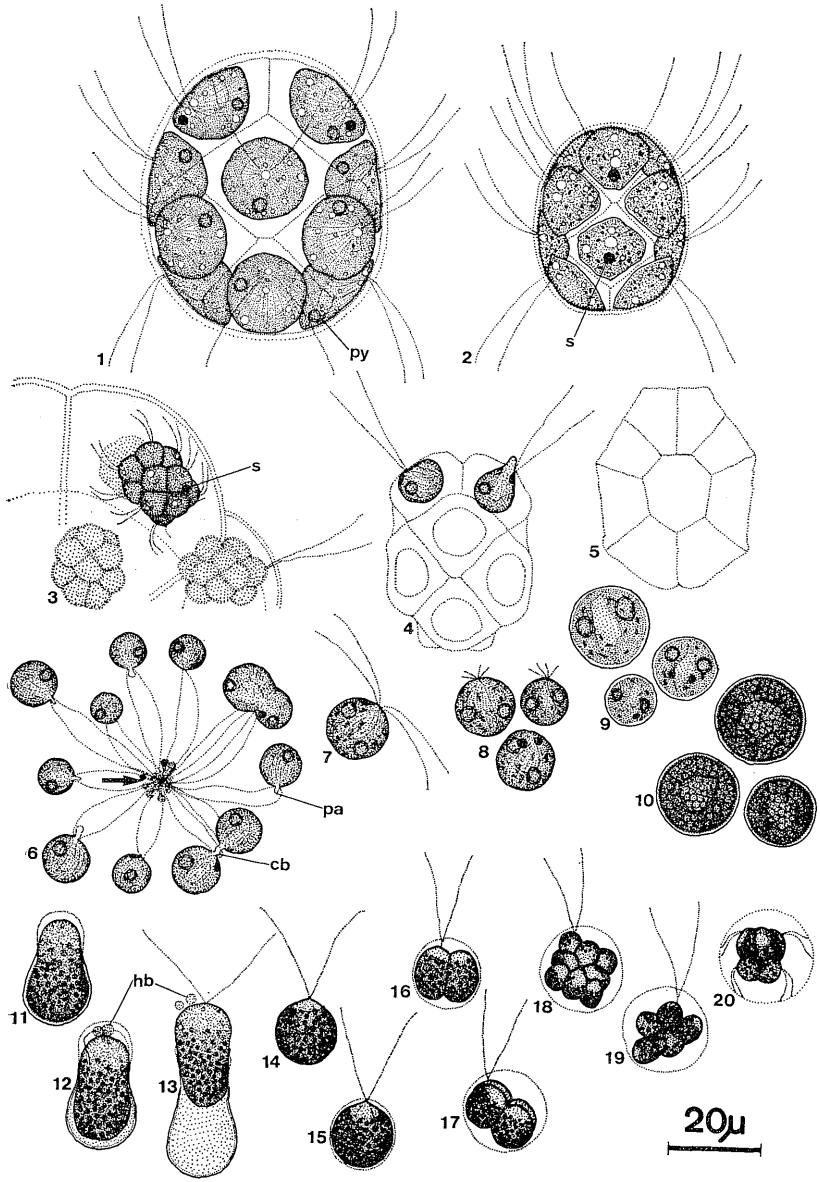
Asexual reproduction. Each cell of the colony formed a daughter colony in the same way. Cell division usually occurred when the cell attained about 20 μm in surface diameter. Four successive divisions formed a 16-celled plakea, and a spherical daughter colony was formed after inversion.

Following the cell divisions, the two flagella remained stuck to one or two of the daughter cells until the spherical colony was formed. Many contractile vacuoles were shared out among the daughter cells. The stigma, when present, remained attached to one of the daughter cells and at last was placed in one of the cells in the three posterior whorls of the daughter colony (Fig. 3). The pyrenoid of the chloroplast, when present, was placed in one of the daughter cells, but gradually became indistinct.

In the late stage of the inversion, each daughter cell began to project two flagella of equal length. As a result, a 16-celled compact colony, which measured 16-20 μm in length, was formed in each keystone-shaped space of the parental gelatinous matrix (Fig. 3; Pl. III F). The daughter colony then swam away from the parental gelatinous matrix. Stigmata developed in the anterior four cells as the colony became bigger, giving the typical shape of *Volvulina*. It took 3 ½-4 hr from the first cell division to the release of the daughter colony from the parental gelatinous matrix.

Sexual reproduction. The strains used in this study were heterothallic, and the mating reaction usually occurred one or two days after the mixing of the colonies. The cells of the colonies participating in the mating reaction were usually hemispheric to spheric in shape.

The first step in the mating reaction was colony clumping (Pl. III G). As the colonies were clumping, all the cells of the colony were released from the gelatinous matrix (Fig. 4; Pl. III H). These cells functioned as gametes. The gametes had the same forms as the vegetative cells except for their spherical shapes and a transparent papilla at the base of the flagella (Fig. 6; Pl. III J). These papillae were varied in size and could not be recognized even with a phase contrast microscope in some gametes.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The gametes soon aggregated in a clump with their flagellar tips sticking to one another (Fig. 6; Pl. III K). Conjugation of the gametes occurred in this clumping. Two of the gametes, whose sizes might or might not be the same, connected with their papillae (Pl. IV L), and the fusing of their bodies proceeded laterally (Fig. 6; Pl. IV M). The zygote separated from the clumping group, when the fusing was nearly completed, leaving the tips of its flagella (arrow, Fig. 6). The zygote was spheroidal in shape and had four flagella, two pyrenoids and stigmata (Fig. 7; Pl. IV N). It took about 1 hr from the beginning of the colony clumping to the formation of the quadriflagellate zygote. This motile zygote soon settled at the bottom of the container, shortened its flagella and entered a dormant period (Fig. 8; Pl. IV O). It secreted a cell wall during the following days (Fig. 9; Pl. IV P) and became reddish brown in colour after about one week (Fig. 10; Pl. IV Q). This matured zygote measured 9–21 μm in diameter.

The zygotes enclosed with nitrogen gas in a tube usually began to germinate within a day of the transfer from darkness to the usual illumination. In the first stage, one part of the zygote wall became distended into a thin-walled protuberance, into which the reddish brown content (gone cell) projected two flagella (Figs. 11, 12). In this space, hyaline bodies were observed (Fig. 12; Pl. IV R). These bodies are considered to be the degenerate products of meiotic division. Next, the thin-walled protuberance ruptured and the biflagellate gone cell escaped, leaving its empty wall (Fig. 13; Pl. IV S). This gone cell was spheroidal in shape (Fig. 14; Pl. IV T) and formed a gelatinous envelope around itself (Fig. 15). About 2 hr after the escape, the gone cell

Figs. 1–20. *Volvulina steinii* Playfair. 1: 16-celled matured colony in four-day-old culture. Note pyrenoid (py) in the brim of bowl-shaped chloroplast of each cell. 2: 16-celled colony in one-day-old culture, cells lacking pyrenoids. Note stigma (s) in one of cells in the third whorl. 3: Daughter colony in parental gelatinous matrix. Note biflagellate cells and parental stigma (s) in one of the cells. 4: Gamete release. 5: Optical section of residual gelatinous matrix after escape of gametes. 6: Gamete clumping and conjugation of gametes. Note papillae (pa) and cytoplasmic bridge (b). Arrow indicates residual flagellar tips of gametes. 7: Quadriflagellate zygote. 8: Zygotes resting with their flagella shortening. 9: Green zygotes with walls and pyrenoids. 10: Reddish brown matured zygotes. 11–13: Germinating zygote. 11: Initial stage of zygote germination. 12: Gone cell projecting two flagella into space formed by thin-walled protuberance. Note hyaline bodies (hb) in this space. 13: Biflagellate gone cell escaping from zygote wall. 14: Biflagellate gone cell. 15: Gone cell with gelatinous envelope. 16–20: Gone colony formation. 16: 2-celled plakeal stage. 17: 4-celled plakeal stage. 18: 8-celled plakeal stage. 19: Inversion stage of 8-celled plakea. 20: 8-celled gone colony in gelatinous envelope.

divided to form a gone colony, though the gelatinous envelope containing the daughter cells swam with the two flagella provided the gone cell until the late stage of the inversion (Figs. 16-19). The process of this gone colony formation was essentially the same as that of daughter colony formation in the asexual reproduction. As a result, a 4-, 8- or 16-celled gone colony was formed in the gelatinous envelope common to the gone cells (Fig. 20; Pl. IV W). The reddish brown granules in the zygote remained in the gone colony, but vanished gradually after the release from the gelatinous envelope.

Discussion My result agreed, to some extent, with that of the cultural studies on *Volvulina steinii* by Pocock (1953), Stein (1958) and Carefoot (1966), except for the presence or absence of pyrenoid of the chloroplast in the vegetative cell and zygote germination. Although these three authors did not mention the formation of an unquestionable pyrenoid in the vegetative cell, I clearly detected it not only in the present strains but also in the three strains which Carefoot (1966) used (FA-4, SC-22 and C2-13), though his strains began to show observable pyrenoids in older cultures. The pyrenoids of his strains appeared in four- to seven-day-old cultures growing in synthetic medium or in those five to 13 days old growing in soil-water-pea medium. In both Carefoot's and the present strains of *V. steinii*, however, usually a single pyrenoid developed in the brim of the bowl-shaped chloroplast as the culture aged (Fig. 1; Pl. III B). Such pyrenoid development in the vegetative phase of *V. steinii* was reported by Korshikov (1938) with his natural collection. It is considered, therefore, that the species *V. steinii* has the potentiality of forming usually a single pyrenoid in the brim of the bowl-shaped chloroplast in the vegetative cell.

The only observation on zygote germination of the genus *Volvulina* reported by Carefoot (1966) with *V. steinii* is different from that of the present study. He said that the zygote wall disintegrated and did not remain as an empty hull. Furthermore, he did not mention about the gelatinous envelope in which the gone colony is formed. In the present study, however, the gone cell escaped from the zygote wall, which remained as an empty hull (Fig. 13; Pl. IV S), and the gone colony was formed in the gelatinous envelope of the gone cell (Figs. 15-20; Pl. IV U-W). With regard to these two characteristics of zygote germination, however, the results of the present study are essentially the same as those reported in several species of the colonial Volvocales, *i.e.*, *Pandorina morum* Bory (Nozaki & Kasaki 1979), *P. unicocca* Rayburn et Starr

(Rayburn & Starr 1974; Nozaki 1981), *Platydorina caudata* Kofoid (Harris & Starr 1969) and *Volvox rousseletii* West (Fritsch 1935).

The asexual reproduction of *Volvulina steinii* is essentially the same as that of *Pandorina morum* (Nozaki 1980) but different from that of *P. unicocca* as well as of *Eudorina* and *Pleodorina* (Nozaki 1981) with regard to the structure of the parental gelatinous matrix and the form of the new flagella projection of daughter cells. It may be indicated, therefore, that there is a close phylogenetic relationship between *Volvulina steinii* and *Pandorina morum*.

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Explanation of plates III-IV

Pl. III. *Volvulina steinii* Playfair. A-B, G: Non-stained. C-F: Stained with haematoxylin. H-K: Observed by phase contrast microscope. A: Surface view of 16-celled colony in two-day-old culture. Note stigmata (s) and contractile vacuoles (v). B: 16-celled colony in four-day-old culture, each cell having a single pyrenoid (py) in the brim of bowl-shaped chloroplast. C: Surface view of 16-celled colony showing flagella (f) and structure of gelatinous matrix. D: Optical section of 16-celled colony showing structure of gelatinous matrix. Note a small hollow in the centre. E: 8-celled plakeal stage in asexual reproduction, showing structure of parental gelatinous matrix. F: 16-celled daughter colonies, each being placed in a keystone-shaped space of parental gelatinous matrix. G: Colony clumping. H: Gamete release. I: Surface view of residual gelatinous matrix after escape of gametes. J: Biflagellate gamete showing papilla (pa) at the base of the flagella. K: Gamete clumping. Arrow indicates residual flagellar tips of gametes.

Pl. IV. *Volvulina steinii* Playfair. L-N, T: Observed by phase contrast microscope. O-S: Observed by audinary light microscope. U-W: Ink preparation. L: Gametes beginning to fuse, showing cytoplasmic bridge (cb) formed by mutual papillae. M: Late stage of conjugation of gametes. N: Quadriflagellate zygote. O: Aplanozygotes before secretion of walls. P: Two-day-old zygotes with walls. Q: Reddish brown matured zygotes. R: Germinating zygote. Note hyaline body in thin-walled protuberance (arrow). S: Empty wall after escape of gone cell. T: Biflagellate gone cell. U-W: Gone colony formation. Note gelatinous envelope surrounding plakea and gone colony. U: 2-celled plakeal stage. V: 4-celled plakeal stage. W: 16-celled gone colony.

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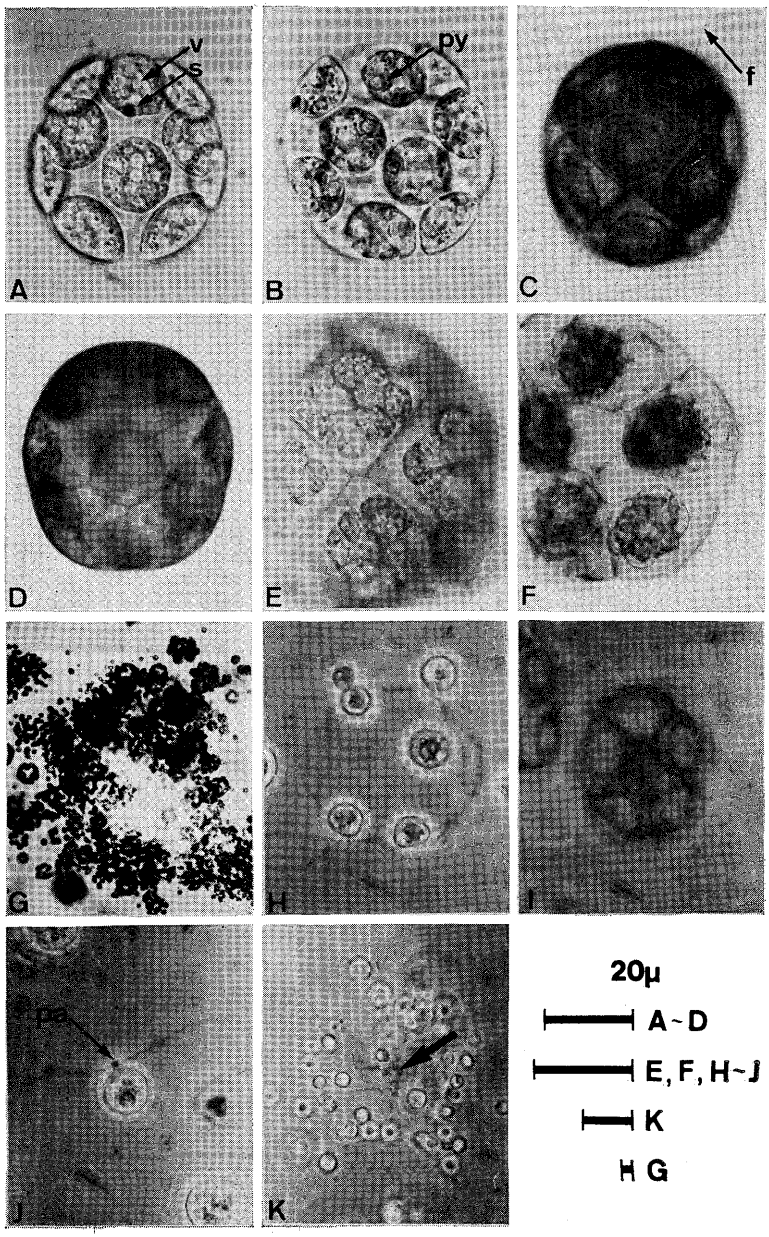
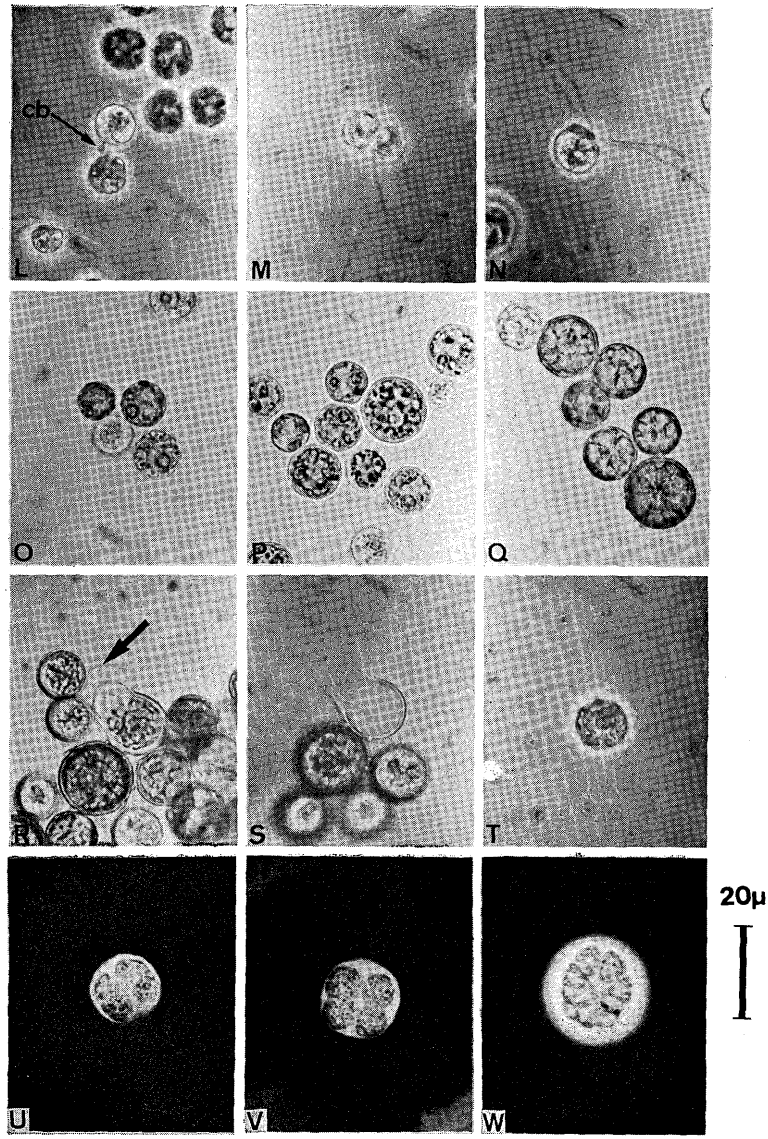
本邦産の *Volvulina* 属に関する詳細な報告はいまだない。筆者は神奈川県葉山町長柄にある水田より採取した *Volvulina steinii* Playfair の形態と生殖の過程を培養条件下で詳細に観察した。その結果は Pocock (1953), Stein (1958), Carefoot (1966) の観察結果と基本的には一致したが, vegetative phase のピレノイドと接合子の発芽に関しては異なる結果を得た。今回, 培養の age が進行すると *V. steinii* の井型の葉緑体の縁の部分に通常1個のピレノイドが形成される事が確認された。この形質は Carefoot (1966) が用いた *V. steinii* の株にも認められたので, 本種が有する特徴と考えられる。また, 接合子の発芽において gone cell は接合子の壁より2鞭毛をつけてぬけ出し, 外側にゼラチン様の膜をつくる。gone colony はその後 gone cell からひきついだ2鞭毛で泳ぎながらゼラチン様膜の中で細胞分裂をくり返す。

□佐竹義輔・大井次三郎・北村四郎・亘理俊次・冨成忠夫（編）: **日本の野生植物 草本** (Satake, Y., J. Ohwi, S. Kitamura, S. Watari & T. Tominari (eds.): Wild flowers of Japan, herbaceous plants (including dwarf subshrubs) 259 pp. pls. 224. 1981. 平凡社 ¥13,000. 待望久しい著作である。冨成忠夫氏の植物写真集がでるとは大分評判であったが, それが今度姿を表わしたといえる。日本全土の高等植物の中で草本約2,800種を一々写真にとり, 4～5点ずつ1ページに納め, 本文8ページ毎にプレート8ページを挿入してまとめてある。本文は合弁花類, 離弁花類, 及び単子葉類として3冊にまとめ, 科の排列はエングラーの Syllabus (1964) に依り, まず科の特徴, 各属の検索表, それに属毎の記載, 種の記載と並べてある。記載についても, 写真についても大勢の学者や専門家がタッチしているので中々充実している。アメリカで出版されたカラー図譜に刺激されての出版というが, それとは数等の素晴らしさを持っている。草本を主としているが, 中にはツツジ科のようにツガザクラ等一見草本に見えるものも含んでいて便利である。殊に写真は全部カラーで, 主に開花期を基準にしているが, 葉の面白い姿や果実等を折に触れて写していて, このプレートをみるだけでも中々に役立つ。写真は背景と被写体との混淆がとかく問題になるが, その点でも大変に苦心している。この大冊の出現を祝し, その完成を祈るものである。 (前川文夫)



H. NOZAKI: *Volvulina steinii*



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