FURTHER STUDIES ON *CIRRULICARPUS GMELINI (GRUNOW) TOKIDA ET MASAKI*

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Cirrulicarpus Gmelini was first recognized as an independent taxon by Grunow (1867, p. 72) who placed it in the genus ***Kallymenia.*** Yendo (1915) transferred the species to ***Erythrophyllum,*** where it remained until Tokida and Masaki (1956) founded the new genus, ***Cirrulicarpus,*** for this taxon.

Tokida and Masaki were the first to record fertile plants of ***Cirrulicarpus,*** and they described the tetrasporangiate and cystocarpic specimens. By sectioning the cystocarpic thallus they also observed the carpogonial branch apparatus and a fusion cell. According to Tokida and Masaki there were six cells in the carpogonial branch, linearly arranged in a strong curve so that the basal cell was brought into close proximity with the carpogonium. They suspected that the basal cell served as the auxiliary cell although the carpogonium was not seen fusing with it. A large fusion cell, formed by all the cells of the carpogonial branch was interpreted by Tokida and Masaki to produce gonimoblast cells. Therefore the carpogonial branch system was believed to be a procarp. The sections of ***Cirrulicarpus Gmelini*** illustrated by Tokida and Masaki (figs. 12-17) clearly showed that the carpogonial branch system is located in the inner cortex, and the trichogyne is long and penetrates through the outer cortex. As the cells of the carpogonial branch system enlarge they grow into the medullary region and often occupy a more or less median position inside the thallus.

While the position and organization of the female reproductive apparatus can be observed from sections, it is practically impossible to observe the reproductive structures in their entirety and in isolation from the vegetative cells because the large cells of the female reproductive apparatus are frequently cut and parts of cells or entire cells may be missing from the field. Because the squash technique described by Norris (1957, p. 254) permits entire reproductive structures to be observed, it was used to gain more details about the carpogonial branch apparatus and the development of the gonimoblast in ***Cirrulicarpus Gmelini.*** Norris's squash technique employed a solution of ten percent sodium hydroxide as a softening reagent for the thallus, after which the tissue was washed in distilled water and stained with Mayer's acid haemalum. The specimens used for these preparations were collected by Masaki in Kushiro, Hokkaido on December 4, 1956 (dried

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specimen in the herbarium of the University of Minnesota, fig. 1), and on February 15, 1958 (preserved in 70 percent alcohol in the collection of the University of Minnesota). Since the specimens collected in February had few satisfactory stages in the development of the gonimoblast, and the specimen collected in December had many stages, most of the observations reported here were made on the latter specimen.

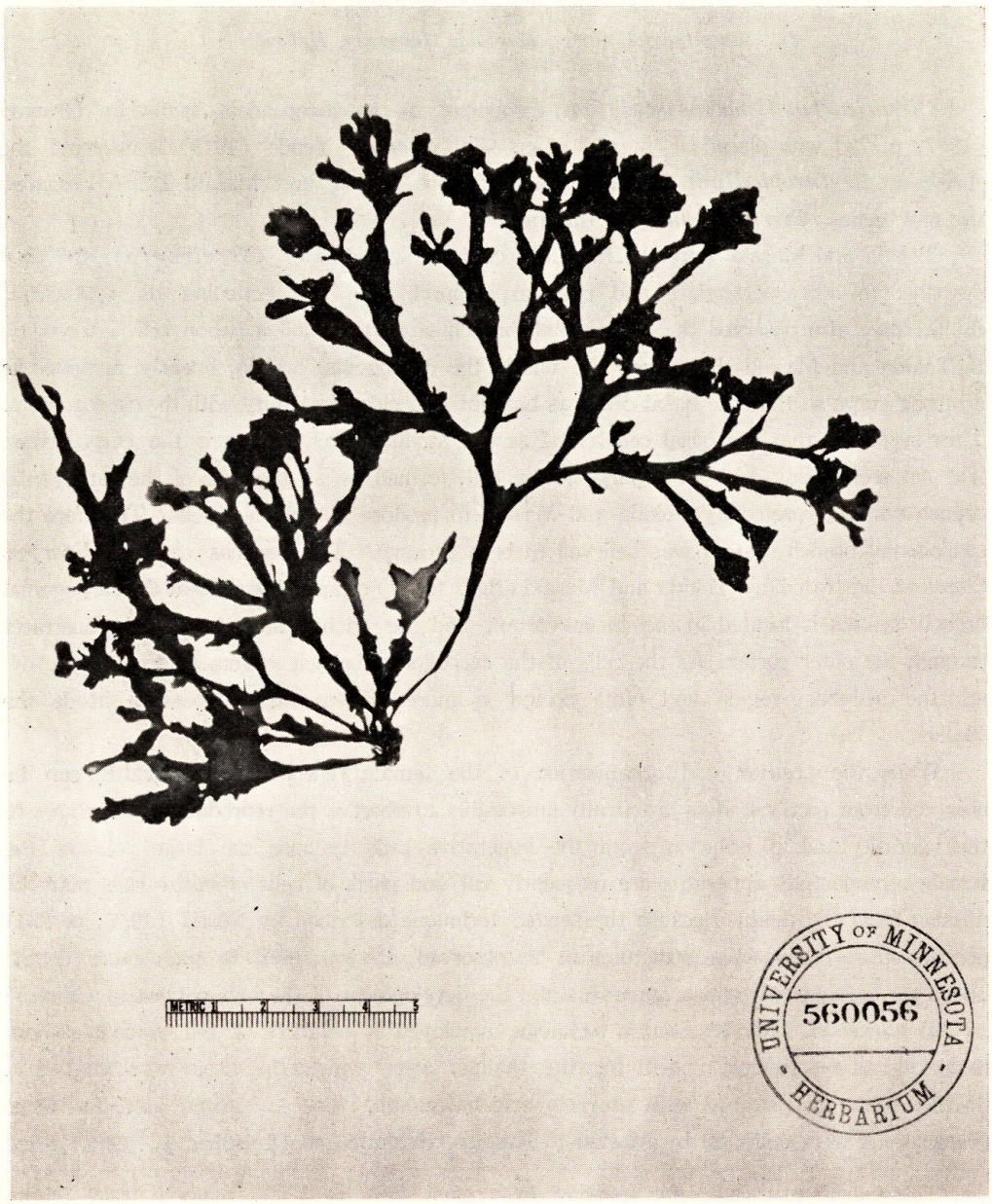


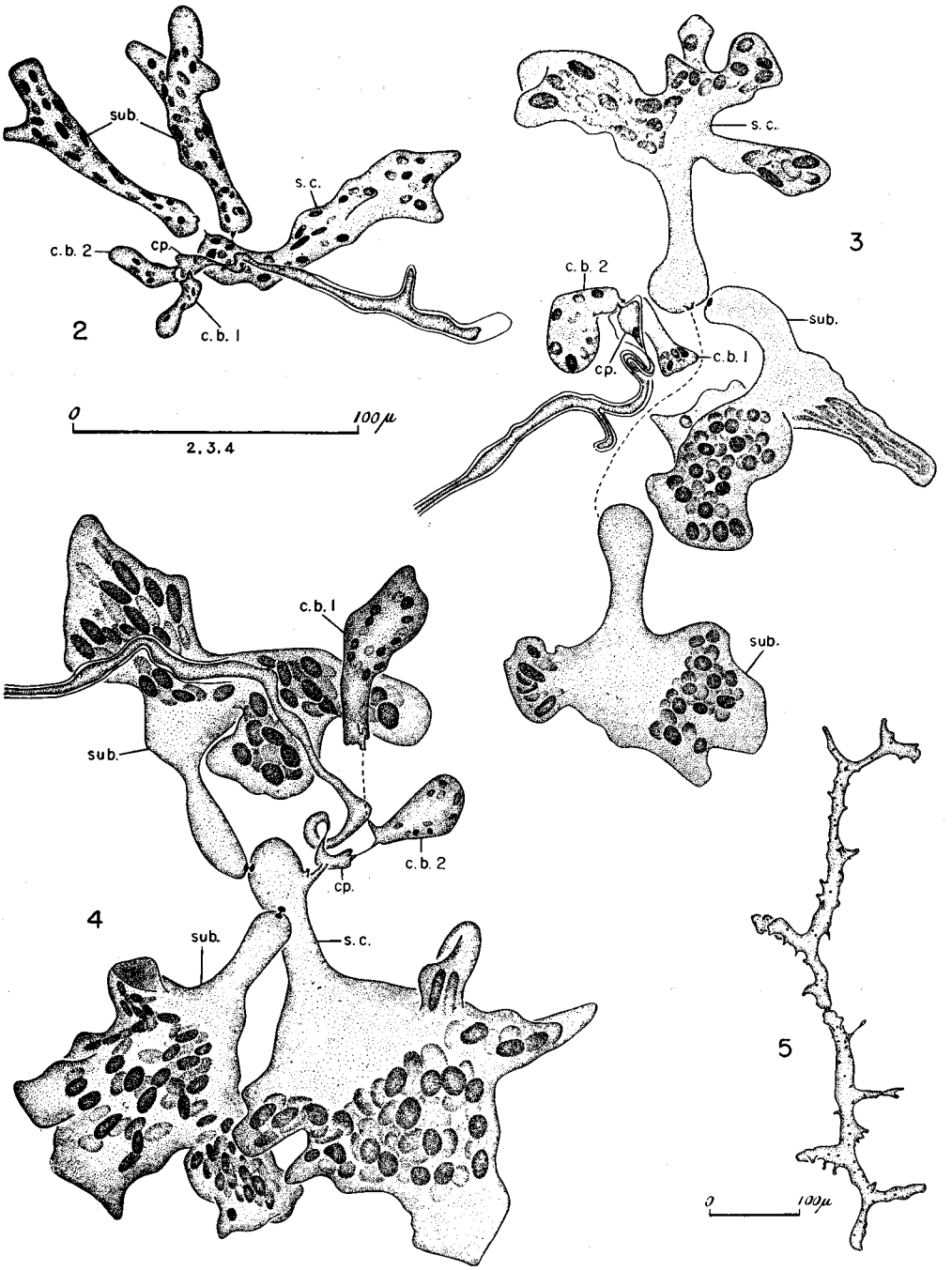
Fig. 1. Female thallus of *Cirrulicarpus Gmelini* collected at Kushiro, 4 Dec. 1956.

From the squash preparations it was found that the carpogonial branch is composed of three cells (fig. 2)*, the terminal one the carpogonium. The trichogyne often has a short branch as in *Erythrophyllum delesserioides;* however, this branch was never fused with any other cell and probably is not involved with the transfer of nuclei to other cells. The first and second cells of the carpogonial branch are similar in size and shape; they are usually oblong-ovate, the narrowed end having the two pit connections very close to one another. Each of these cells usually contains from ten to twenty nuclei. The first cell of the carpogonial branch is connected to a very large cell that often is deeply lobed when mature (fig. 3). This cell is the supporting cell and one of its lobes has several large pit connections where from two to three subsidiary cells in addition to the carpogonial branch are attached. The subsidiary cells are large and often lobed so that they closely resemble the supporting cell; however the supporting cell is slightly larger and more lobed than the subsidiary cells. Each of the subsidiary cells and the supporting cell contain numerous nuclei. As the cells mature they enlarge greatly and the nuclei migrate into the lobes opposite the pit connections. At this stage it is not uncommon to find from 45 to 88 nuclei in each of these large cells.

Stages in the actual fertilization process were not observed, and the nucleus within the carpogonium was seen only a few times. However, in fourteen different female apparatuses the carpogonium was fused with the supporting cell (fig. 4), and in two systems the carpogonium was seen after it had fused with one of the subsidiary cells. It may be hypothesized that the diploid nucleus usually migrates from the carpogonium into the supporting cell, but occasionally it may be transferred from the carpogonium to one of the subsidiary cells.

Subsequent to the transfer of the diploid nucleus into one of the large cells of the carpogonial branch system a fusion cell is formed by the uniting of the subsidiary cells with the supporting cell (fig. 6 & 7). The cells of the carpogonial branch do not join with the fusion cell. Stages in the development of the fusion cell were not evident in the material at hand. The fusion cell enlarges somewhat and at the same time it produces numerous small cells from the ends of the lobes. These small cells elongate rapidly and produce non-septate tubes, the connecting filaments, that radiate from the fusion cell (fig. 6). The connecting filaments grow through the medullary tissue for rather long distances. It seemed that only the tips of the connecting filaments contained an active protoplast, the remaining part of the tube usually appeared to be nearly devoid of protoplasm. Oftentimes vegetative cells were laterally attached to the connecting filaments which may indicate that they provide supplementary nourishment for the growth of the long tubes.

*The illustrations were prepared by Miss Wilma Monserud of the Department of Botany, University of Minnesota.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Diploid nuclei are probably present within the terminal parts of the connecting filaments. When the apices of these filaments come into contact with young, unfertilized carpogonial branch systems they fuse with the supporting cell or one of the subsidiary cells and one or the other of these cells serves as the auxiliary cell. The fusion seems to be a stimulus to the growth of the connecting filament which then enlarges and produces many lobes (fig. 8 & 9). At the same time the connecting filament and the auxiliary cell cut off several large cells that become filled with dense protoplasm (fig. 9). These cells are gonimoblast initials and they divide to produce gonimoblast filaments that radiate throughout the immediate area. Eventually these gonimoblast filaments develop clusters of carposporangia near their apices. The connecting filament may continue to grow through the medulla after diploidization of the auxiliary cell.

According to Tokida and Masaki the cystocarps of *Cirrulicarpus Gmelini* are small elliptical rings that are elongated in the direction of the frond axis. The center of the ring probably was the location of the fusion cell. Since the vegetative cells soon grow into the fusion cell, apparently digesting it, there is little left of the fusion cell by the time the carposporangia have matured. The clear interior region of the ring represents at least part of the length that the connecting filaments grew before they fused with auxiliary cells. As the gonimoblasts from each of the diploidized auxiliary cells developed they fused with one another to form an elliptical ring. Therefore the cystocarps of *Cirrulicarpus Gmelini* are probably compound structures resulting from the development of adjacent gonimoblasts that fused together. Since fertilization and diploidization probably occur only in the young growing parts of the frond, the elliptical shape of the cystocarps seems to be caused by growth of the thallus after diploidization. As was noted by Tokida and Masaki, it is common to observe adjacent cystocarps confluent with one another. In *Cirrulicarpus Gmelini* it is impossible to interpret the cystocarp as the product of one diploidization or even a single fertilization.

The morphology and arrangement of the cells in the monocarpogonial branch apparatus of *Cirrulicarpus Gmelini* emphasizes Tokida's and Masaki's conclusion that it belongs in the Kallymeniaceae. The general morphology of the thallus, the anatomy of the thallus and the monocarpogonial female apparatus with a carpogonial branch of only three cells are characteristics that ally the genus with *Erythrophyllum*. In the Kallymeniaceae, *Erythrophyllum delesserioides* and *Cirrulicarpus Gmelini* are the only

Fig. 2. Carpogonial branch system before fertilization. Fig. 3. Carpogonial branch system, after fertilization. Fig. 4. Carpogonial branch system, after fertilization; the carpogonium has made a connection with the supporting cell. Fig. 5. Two large vegetative cells of the medulla that become filled with a dense proteinaceous material. c.b.1, first cell of carpogonial branch; c.b.2, second cell of carpogonial branch; cp., carpogonium; s.c., supporting cell; sub., subsidiary cells.

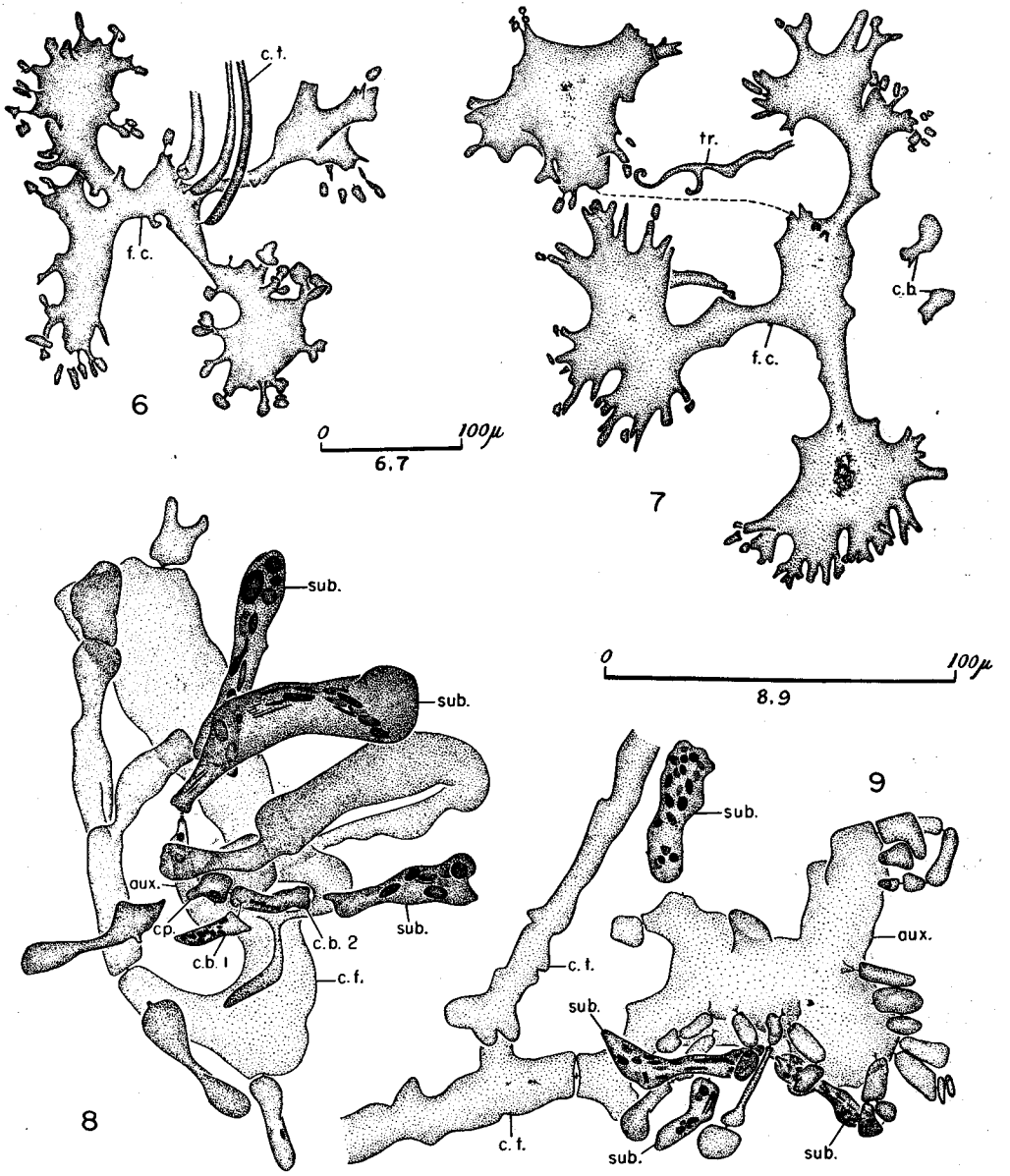

Fig. 6. Fusion cell with three connecting filaments attached to it. Fig. 7. Fusion cell with remnants of the carpogonial branch. Fig. 8. Auxiliary cell system shortly after fusion with the connecting filaments. Fig. 9. Auxiliary cell system, the auxiliary cell has enlarged and is producing gonimoblast initial cells. aux., auxiliary cell; c.b., cells of the carpogonial branch; c.f., connecting filaments; cp., carpogonium; f.c., fusion cell; sub., subsidiary cell; tr., trichogyne.

species that have been investigated in which the first cell of the carpogonial branch does not become part of the fusion cell. Since *Cirrulicarpus Gmelini* does not produce its reproductive organs in specialized papillae as in *Erythrophyllum delesserioides*, there seems to be good justification for maintaining the taxa in separate genera.

Cirrulicarpus exhibits two characteristics that suggest it is probably primitive in relation to other genera in the Kallymeniaceae: 1) a filamentous medulla with little enlargement of sub-cortical cells; 2) a carpogonial branch on the auxiliary cell system. In the latter characteristic it differs from *Erythrophyllum delesserioides* but agrees with *Pugetia fragilissima* (see Norris 1957, fig. 22).

The large cells in the medulla that are filled with a yellowish proteinaceous substance as noted by Tokida and Masaki, may also indicate the primitive position of *Cirrulicarpus* in the Kallymeniaceae, since similar cells are also present in other primitive genera: *Kallymenia, Glaphyrymenia* and *Erythrophyllum*. Ruprecht (1851, p. 267) found cells similar to these in *Crossocarpus lamuticus RUPR*. It is possible that the cells illustrated by Ruprecht (Pl. 14, fig. ac) were parts of carpogonial branch apparatuses rather than vegetative cells. However, Yendo (1915, p. 234) reported "long and fusiform" cells in the medulla of *Crossocarpus lamuticus* that may be similar to those found in *Cirrulicarpus*. In addition to these cells Yendo reported that many smaller "clavate" cells were present. It is possible that the smaller cells may have been parts of female reproductive apparatuses.

Norris interpreted vegetative stellate cells in *Kallymenia reniformis* to have a mechanical function in lending strength to the thallus and resistance to tearing. Although the large medullary cells in *Cirrulicarpus* are seldom stellate, they often branch and are usually attached to one another. A mechanical function as well as a possible function in food reserve or translocation of nutritive substances may be attributed to them. Two of these large cells as they appeared in the squashed preparations are illustrated in fig. 5.

Summary

Additional information is provided on the structure of the carpogonial branch apparatus and development of the gonimoblast for *Cirrulicarpus Gmelini (GRUNOW) TOKIDA ET MASAKI*. The auxiliary cell system is isolated from the carpogonial branch apparatus, but they are identical in structure and each is monocarpogonial. The carpogonial branch consists of three cells. Development of compound cystocarps in *Cirrulicarpus* is considered. The information provided supports Tokida and Masaki in their conclusion that *Cirrulicarpus* belongs in the Kallymeniaceae.

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