Jpn. J. Phycol. (Sôrui) 37: 201–207, September 20, 1989

# Karyology and nuclear DNA content of *Gelidium pusillum* (Gelidiales, Rhodophyta) from North Carolina, USA

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KAPRAUN, D.F. and BAILEY, J.C. 1989. Karyology and nuclear DNA content of *Gelidium pusillum* (Gelidiales, Rhodophyta) from North Carolina, USA. Jpn. J. Phycol. 37: 201–207.

*Gelidium pusillum* (STACKHOUSE) LE JOLIS from coastal North Carolina was found to have 10 bivalents during diakinesis of tetraspore mother cells, indicating a chromosome complement of 2N=20. The DNA-localizing fluorochrome hydroethidine with epi (incident) UV illumination was used to determine relative nuclear DNA in tetrasporophyte cortical cells and tetraspore mother cells, and in gametophyte germling cortical cells. Results from microspectrophotometry provide evidence of nuclear DNA fluctuations correlated with the alternation of haploid and diploid chromosome complements observed in this species' life history.

*Key Index Words: chromosomes—DNA quantification—epifluorescence*—Gelidium—*Rhodophyta.*

As presently circumscribed, the Gelidiales comprise one of the smallest orders of Florideophycidae (HOMMERSAND and FREDERICQ 1988), consisting of a single family, the Gelidiaceae (MAGGS and GUIRY 1987) with nine genera (SANTELICES and STEWART 1985). Five of these genera are monotypic, or are represented by a few species with relatively restricted distributions. Much of the attention devoted to this order has centered on the numerous species of *Gelidium* and *Pterocladia* as they are often conspicuous members of temperate and tropical floras (SANTELICES and STEWART 1985) as well as commercially important sources of agar (GUZMÁN DE PRÓO and DE LA CAMPA DE GUZMÁN 1978, SANTELICES *et al.* 1981, MCLACHLAN 1985).

The most reliable taxonomic feature for distinguishing *Gelidium* from *Pterocladia* is based on cystocarpic structure with *Pterocladia* having a single locule (or two unequally developed locules), while *Gelidium* is bilocular (FAN 1961, KRAFT 1981, AKATSUKA 1986a, HOMMERSAND and FREDERICQ 1988). Unfortunately, female reproductive structures are uncommon or unknown for many of these species (WEST and HOMMERSAND 1981, SANTELICES and STEWART 1985). Consequently, morphological characters of non-cystocarpic plants are routinely used for taxonomic delineations at both the genus and species levels (STEWART 1986, 1974, STEWART and NORRIS 1981, AKATSUKA 1986b, MAGGS and GUIRY 1987).

Need for additional means of delimiting species has been prompted by emerging information that *Gelidium* and *Pterocladia* species produce characteristic agars with distinct commercial applications, as well as the desire to efficiently manage and utilize this natural resource (SANTELICES and STEWART 1985). Elsewhere, cytogenetic studies have provided criteria to distinguish closely related species of red algae including *Porphyra* (MUMFORD 1975, MUMFORD and COLE 1977, KAPRAUN and FRESHWATER 1987), *Gracilaria* (BIRD *et al.* 1982) and *Polysiphonia* (KAPRAUN 1977, 1978). Unfortunately, no karyological information is available for any species of *Pterocladia,* and published data for *Gelidium* is restricted to imprecise chromosome numbers for three species (DIXON 1954, BOILLOT 1963, MAGNE 1964, KANEKO 1966). In contrast, karyological studies of two other Gelidiaceae, *Acanthopeltis japonica* OKAMURA (KANEKO

1968) and *Gelidiella acerosa* (FORSSKÅL) J. FELDMANN et HAMEL (RAO 1974), have shown that careful selection of material and use of appropriate technique can result in precise chromosome numbers as well as evidence of meiosis. Consequently, recently developed cytogenetics methods (KAPRAUN and GARGIULO 1987a,b, KAPRAUN and FRESHWATER 1987) were modified for use with *Gelidium pusillum* (STACKHOUSE) LE JOLIS (=*G. crinale* (TURN.) LAMOUR.), a common member of the local flora in coastal North Carolina (KAPRAUN 1980).

In addition, the DNA-localizing fluorochrome hydroethidine with epi-(incident) UV illumination (KAPRAUN *et al.* 1988) was used to demonstrate fluctuations in nuclear DNA levels associated with meiosis by comparing the C levels of tetrasporophytes, tetraspore mother cells and gametophyte germlings.

## Materials and Methods

*Gelidium pusillum* plants were collected in June 1988 from intertidal rocks at Kure Beach, North Carolina (see KAPRAUN 1980 for habitat description and location map). Fertile branch tips bearing tetrasporangia were excised, cleaned of epiphytes and debris, and placed in petri dishes with 20 ml enriched seawater medium (VSE) (FRESHWATER and KAPRAUN 1986). Mature tetraspores were released within 6 hr after which the fertile branches were removed and fixed at 24:00 in 3 : 1 absolute ethanol-glacial acetic acid (AUSTIN 1959). Tetraspores were incubated at 22°C, 100 $\mu E \cdot m^{-2}s^{-1}$ photon flux density and 12 : 12 LD for 14 days. Resultant germlings were fixed as above. Material fixed for chromosome counts was treated and stained in 2% aceto-orcein (KAPRAUN and MARTIN 1987). Documentation was made by microphotographs and by viewing 35 mm Kodak Plus-X film with a 48X microfiche reader and tracing the projected images (KAPRAUN and FRESHWATER 1987).

Fixed material for measurement of nuclear DNA was stained with the DNA-localizing fluorochrome hydroethidine for 10 min and then destained in phosphate buffer (PBS) for 48 hr prior to examination (KAPRAUN 1989). Epi-fluorescence data were standardized to the average intensities ($I_f$) of telophase (2C) nuclei in tetrasporophyte cortical cells (KAPRAUN *et al.* 1988) and the results analyzed and presented in histograms (GOFF and COLEMAN 1984). Observations and photomicrographic documentation were made with brightfield and epi-(incident) UV illumination using an Olympus BH2-RFK microscope and exciter filter BP-545, dichroic mirror DM-580 and barrier filter 0–590 which are specific for hydroethidine emissions (KAPRAUN *et al.* 1988).

## Results and Discussion

Chromosome numbers for seven taxa of Gelidiales in the present and previously published investigations are given in Table 1. In *Gelidium pusillum,* 10 bivalents were observed in meiotic metaphase (diakinesis) of tetraspore mother cells (Fig. 1). The haploid chromosome complement of 1 N=10 appears to include six chromosomes which are conspicuously larger than the other four (Fig. 1). Tetrasporophyte cortical and medullary cell preparations inlcuded many late prophase and metaphase mitotic nuclei in which 17–18 of the 20 chromosomes present could be observed (Fig. 2). Unfortunately, the tendency for the nuclear envelope to remain intact and confine chromosomes during mitosis precluded accurate counts in these cells.

The basic chromosome complement for the Gelidiales appears to be 1X=5 (KANEKO 1968), with diploid (2 N) numbers of 10, 20 and 30 (Table 1) representing a polyploid series. The reported aneuploid number of N=4 in *Gelidiella acerosa* (RAO 1974), if correct, suggests that this taxon may be distinct from *Gelidium* species. It would be a matter of great interest to confirm this report and to obtain karyological details for additional *Gelidiella* species in light of recent evidence that questions the validity of the criteria used to delimit *Gelidiella* from *Gelidium* (MAGGS and

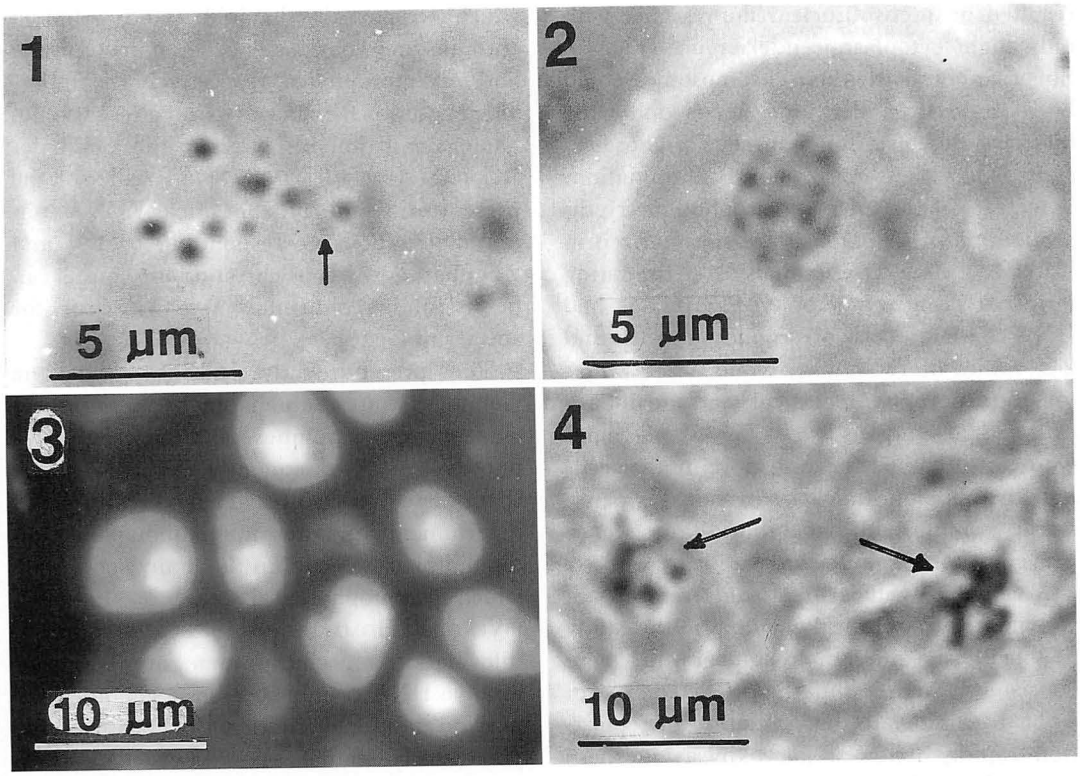
Table 1. Chromosome numbers in species of Gelidiaceae.

| Species | Chromosome number* | | Reference |
|---|---|---|---|
| | 1 N | 2 N | |
| *Acanthopeltis japonica* Okamura | 15 | 30 | Kaneko 1968 |
| *Gelidiella acerosa* (Forsskal) J. Feldmann et Hamel | 4 | 8 | Rao 1974 |
| *Gelidium latifolium* (Grev.) Bornet et Thuret | 5 (4– 5) | 10 ( 9–10) | Dixon 1954 |
| *Gelidium latifolium* (Grev.) Bornet et Thuret | | 20 (c.18) | Boillot 1963 |
| *Gelidium latifolium* (Grev.) Bornet et Thuret var. *luxurians* (Crouan) Hamel et Feldmann | | 30 (25–30) | Magne 1964 |
| *Gelidium pusillum* (Stackh.) Le Jolis | 10 | 20 | Present study |
| *Gelidium sesquipedale* (Clemente) Thuret (as *G. corneum* (Huds.) Lamour.) | 5 (4– 5) | 10 ( 9–10) | Dixon 1954 |
| *Gelidium vagum* Okamura | 10 (7–10) | | Kaneko 1966 |

* Ploidy levels were not indicated in all references.

Guiry 1987).

Microspectrophotometry with DNA-localizing fluorochromes has been used extensively for quantitative determination of nuclear DNA in algae (Al-Kubaisy *et al.* 1981, Coleman 1982, Goff and Coleman 1984, 1986,




Figs. 1 & 2. Tetrasporophyte nuclei following aceto-orcein staining. Fig. 1. Meiotic metaphase (diakinesis) with 10 bivalents in a tetraspore mother cell. Arrow indicates additional chromosome below focal plane. Fig. 2. Cortical cell showing early mitotic prophase with 17–18 of the 20 chromosomes present.

Figs. 3 & 4. Tetrasporophyte nuclei following hydroethidine staining. Fig. 3. Cortical cell nuclei visualized with episcopic UV illumination. Fig. 4. Tetraspore mother cell with meiotic prophase II nuclei (arrows) visualized with bright field illumination.

Table 2. Fluorescence intensity ($I_f$) mean±SD for *Gelidium pusillum* phases.

| | n | 1 C | 2 C | 4 C |
|---|---|---|---|---|
| Tetrasporophyte cortical cells | 20 | | 50±5 | |
| | 20 | | 51±4 | |
| | 7 | | 53±4 | |
| | 20 | | | 101±4 |
| | 20 | | | 98±6 |
| | 20 | | | 96±8 |
| Mature tetraspores | 10 | | 51±7 | |
| Germinating tetraspores and gametophyte germlings | 30 | | 49±8 | |
| | 14 | 27±4 | | |
| | 20 | 32±4 | | |
| Mean $I_f$ | | 29 | 51 | 98 |

SCHNETTER *et al.* 1984, KAPRAUN *et al.* 1988). In the present study, hydroethidine staining for periods as brief as 10 min followed by destaining in PBS buffer for 2-3 days at 4°C resulted in intense nuclear fluorescence with only slight cytoplasmic interference (Fig. 3). Surprisingly, individual chromosomes could be observed in dividing nuclei following hydroethidine staining under bright field (Fig. 4) as well as episcopic UV illumination.

Results of the microspectrophotometry investigation of *G. pusillum* are summarized in Table 2. Mean DNA values for germinating tetraspores and gametophyte germlings had DNA content levels corresponding to 1 C and 2 C values of 1N nuclei, while DNA values for tetrasporophyte cortical and medullary cells had 2 C and 4 C levels (Fig. 5). $I_f$ values were not obtained for tetraspore mother cells and their meiotic nuclear divisions as dense starch granules and background fluorescence prevented precise nuclear readings.

Mean DNA values for 2 C nuclei closely approximate 50% of the 4 C values in this study (Table 2). $I_f$ values for 1 C nuclei which were consistently higher than the predicted 50% of 2 C nuclei indicate that initiation of replication rapidly followed karyokinesis in these cultured germlings.

The assumption that members of the Gelidiales possess a *Polysiphonia*-type life history is based primarily on reports of isomorphic gametophytes and tetrasporophytes in nature (WEST and HOMMERSAND 1981), and has been demonstrated in culture for only one species, *Gelidium coulteri* HARVEY (MACLER and WEST 1987). Cytogenetic investigations which reported haploid and diploid chromosome complements for gametophytes and tetrasporophytes, respectively, have provided additional evidence for an alternation of haploid and diploid phases in the Gelidiales (KANEKO 1968, RAO 1974). Results of the present study indicate that nuclear DNA content fluctuations corresponding to 1 N and 2 N phases as well as chromosome counts can be useful in confirming a sexual cycle for species of Gelidiales.

The present study indicates that the Gelidiales are amenable to improved cytogenetic techniques and microspectrophotometry. Consequently, it is concievable that these procedures could be used to determine if an alternation of haploid and diploid phases occurs in the many species for which gametophytes are rare or unknown (WEST and HOMMERSAND 1981). In addition, information for chromosome numbers and relative DNA content per basic genome may provide additional criteria for delineating morphologically similar taxa. For example, a previous investigation of *Codium* (Chlorophyta) in the North Atlantic demonstrated that four superficially similar erect species could be distinguished by a combination of chromosome numbers and interspecific

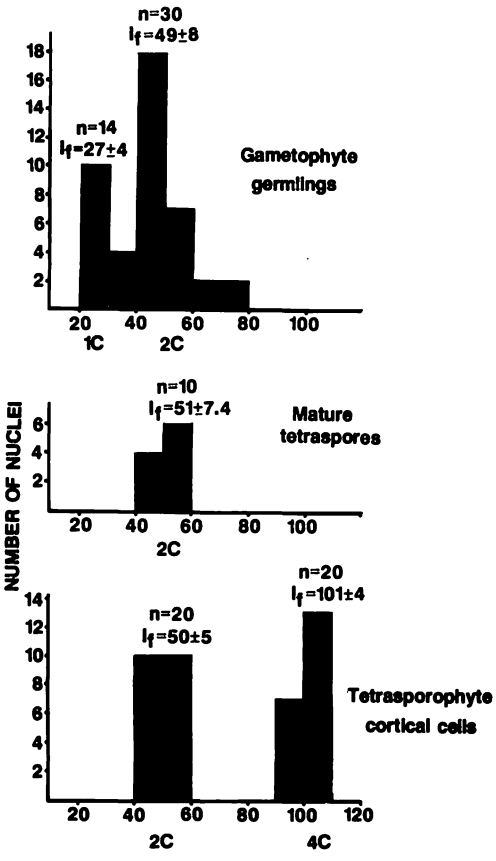



Fig. 5. Frequency distribution of relative DNA values for tetrasporophyte and gametophyte nuclei following hydroethidine staining. n = number of nuclei, $I_f$ = fluorescence intensity mean ± SD.

DNA contents (KAPRAUN *et al.* 1988).

Finally, preliminary studies of genetic modifications in the agar-producing red alga *Gracilaria tikvahiae* MCLACHLAN suggest that autopolyploidization can result in improved mariculture stock (VAN DER MEER 1983, VAN DER MEER and PATWARY 1983). However, karyological criteria for successful autopolyploidization include a suboptimal (or low) number of small chromosomes (LEVAN 1945). Consequently, *Gracilaria* species which have N = 24 or N = 32 (MAGNE 1964, MCLACHLAN *et al.* 1977, BIRD and MCLACHLAN 1982, BIRD *et al.* 1982) are only marginally suited for this form of genetic modification (VAN DER MEER and PATWARY 1983). In contrast, haploid chromosome complements of 1 N = 5 and 10 in *Gelidium* species (Table 1) suggest that these taxa may be more likely candidates for genetic improvement by autopolyploidization.

## Acknowledgements

The authors gratefully acknowledge financial support for this research from the National Science Foundation (BSR-RUI 88-17846), the Cottrell Foundation (C-2546) and the North Carolina Center for Biotechnology. CMSR Contribution No. 006.

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## Donald F. KAPRAUN・J. Craig BAILEY：米国ノースカロライナ沿岸より得た紅藻テングサ目の一種 *Gelidium pusillum* の核学並びに核 DNA 含有量

米国ノースカロライナ沿岸より得た紅藻テングサ目の一種 *Gelidium pusillum* について核学的研究を行ない，四分胞子母細胞のディアキネシス期では10個の二価染色体を有することを認めた。また，フロロクロム・ハイドロエチディンで染色し，紫外線を投射し，蛍光顕微分析を行って，四分胞子体の皮層細胞と四分胞子母細胞並びに配偶体の皮層細胞について相対的な DNA 量を調べた。その結果から，本種が複相と単相の世代を有していることが示唆された。(Department of Biological Sciences, University of North Carolina, Wilmington, North Carolina 28403, U.S.A.)