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Chromosome numbers in species of *Porphyra* from Nova Scotia, Canada

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Four species of *Porphyra*, *P. leucosticta*, *P. umbilicalis*, *P. linearis* and *Porphyra* sp., from Nova Scotia were studied cytologically. Chromosome numbers were n=3, 2n=6 in *P. leucosticta* and *Porphyra* sp., and n=5, 2n=10 in *P. umbilicalis* and *P. linearis*. In formation of spermatia, one of the chromosomes at prophase was longer than the other two in *Porphyra* sp., and two of the five were longer in *P. umbilicalis*. In culture, dwarf leafy plants of *P. leucosticta* had the haploid number of chromosomes, and the conchocelis of *P. umbilicalis* had the diploid number of chromosomes.

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Four species of *Porphyra, P. leucosticta* THUR. in LE JOL., *P. linearis* GREV., *P. miniata* (C. AG.) C. AG., and *P. umbilicalis* (L.) J. AG. have been recorded form the coast of Nova Scotia, Canada (EDELSTEIN and MCLACHLAN, 1965), elsewhere in eastern Canada (SOUTH, 1976), and the northwestern Atlantic (TAYLOR, 1957). Amongst these species, chromosome numbers have been determined both for *P. miniata* (KITO *et al.*, 1971) and *P. linearis*, although in the latter the number given has varied from n=2 (DANGEARD, 1927), n=4 and 2n=8 (KITO *et al.*, 1965; MAGNE, 1952; GIRAUD and MAGNE, 1968). The present paper reports on chromosome numbers and cytological observations on the four species of *Porphyra* from Nova Scotia.

Methods

Following collection, thalli were transferred to the laboratory in polybags on ice. These plants were maintained at 5°C in filtered seawater until fixation. Some thalli of both *P. leucosticta* and *P. umbilicalis* were held under these conditions until maturation and release of spores occurred. Spores released were inoculated into 150 mm diameter Petri dishes containing filtered seawater with several glass slides, and incubated for about two weeks on the sill of a north-facing window. Both leafy thalli and sporelings were fixed in alcohol: acetic acid (3: 1) and stained with Wittmann's solution (WITTMANN, 1965)

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Observations

1. *Porphyra leucosticta* THUR. in LE JOL.

My observations in the field at Gulliver Cove, Digby Co. were from October to April, and this alga was always present in abundance on rocks and large boulders in the upper intertidal area (EDELSTEIN *et al.*, 1970). This species is monoecious as has been reported by others (TAYLOR, 1957; CONWAY, 1964; COLL and DE OLIVEIRA FILH, 1976; KORNMANN, 1961), and mature plants were obtained from November to February.

Dividing nuclei in somatic cells of young

plants collected in late October showed three chromosomes. In plants collected from November to February, numerous dividing nuclei were observed in formation of spermatia (Pl. I, A-C). Chromosomes in these cells, from late prophase to early metaphase, occasionally assumed a rugged form, being considerably larger in size (Pl. I, B). In specimens collected in late November, six chromosomes were noted, both in the first (Pl. I, D-F) and second divisions leading to formation of carpospores.

In culturing *P. leucosticta*, KORNMANN (1961) reported that two types of plants, dwarf leafy thalli and conchocelis filaments, arose from carpospores. EDWARDS (1969) suggested that the type of progeney from carpospores was controlled by the period of light. In my cultures carpospores usually developed into dwarf plants, but in several instances spores from plants collected in November and December gave rise to dwarf plants, together with a few filaments of the conchocelis phase. In formation of dwarf plants, dividing nuclei were frequently found in the one- or two-celled stages of development, and these always had three chromosomes (Pl. I, G-I, L). However, dividing nuclei were never observed in the conchocelis filaments, and their complement of chromosomes remains to be determined. It is reasonable to consider that dwarf plants develop from what has been referred to as neutral spores, whereas conchocelis filaments arise from carpospores following fertilization of the carpogonium. Therefore, it is especially unfortunate that I was unable to determine the chromosome number in both types of progeny when they occurred in culture simultaneously.

2. *Porphyra umbilicalis* (L.) J. AG.

The occurrence of this species in Nova Scotia during winter has been discussed by EDELSTEIN and MCLACHLAN (1965), who distinguished three morphological forms (typical form, mid-level form, and high-level form) associated with tidal levels.

In the northwestern Atlantic, *P. umbilicalis* has been considered both monoecious and dioecious (TAYLOR, 1957). In Helgoland, KORNMANN (1961) considered this species to be dioecious, whereas CONWAY (1964) stated that β-spores were not always present; when β-spores were present they formed a colourless fringe outside the large, pigmented α-spores. In my experience, plants taken in late November at Herring Cove, Halifax Co., were monoecious, male portions of the thallus becoming obvious earlier than female portions.

Leafy thalli were collected in late November from the mid-littoral zone at Gulliver Cove, Digby Co., and from the low littoral zone at Herring Cove, Halifax Co., and the Container Pier in Halifax Harbour. At this time thalli obtained from Gulliver Cove and the Container Pier contained relatively few spores. Nevertheless, five chromosomes were observed at metaphase in cells leading to formation of spermatia. Plants from Herring Cove were fully reproductive. In these plants too, cells leading to formation of spermatia had five chromosomes (Pl. II, A-C), and cells forming carpospores had ten chromosomes (Pl. II, D-E). In the former at prophase, two of the five chromosomes were noticeably longer (Pl. II, A-B).

Cultures starting with carpospores were initiated in late November from plants collected at Herring Cove, and equivalent numbers of dwarf plants and conchocelis filaments developed from these spores. About five days after inoculation, the conchocelis filaments started to bleach. Dwarf plants were not affected similarly, and several days later put forth rhizoids.

I was able to observe nuclear division in spores forming the conchocelis, but not in those forming the dwarf plant. In early prophase, small chromatin granules occurred within the nuclear cavity (Pl. II, F), this stage being rather prolonged. The number of chromosomes counted in late prophase and metaphase was eight to ten (Pl. II, G). Usually the spindles were clearly visible at anaphase.

Formation of the germinating tube of the

conchocelis was usually observed while the nucleus was in telophase. Following nuclear division, almost all of the cytoplasm including the chromatophore and one of the daughter nuclei moved into the protuberance, and the first cell of the filament was formed by cytokinesis. The other daughter nucleus remained within the original spores, which otherwise appeared empty. This nucleus was always in early prophase, being stained darkly and containing chromatin granules. In some instances this nucleus was observed again to divide within the cell of the original spores.

In cells of the filamentous conchocelis, the nucleus was usually situated within the central portion of the cell, and assumed an ellipsoidal form. The nucleus entering into early prophase became spherical and increased in size rapidly. Occasionally I observed these nuclei to have two to five nucleoli (Pl. II, H-I). Out of twenty-one metaphase encountered in cells of the conchocelis filament, it was possible to observe eight to ten chromosomes in eight of these nuclei (Pl. II, J-L).

3. *Porphyra linearis* GREV.

In Nova Scotia *P. linearis* has been studied both in culture (BIRD *et al.* 1972) and in the field (BIRD, 1973). BIRD (1973) has indicated that potentially this species is monoecious.

Plants of *P. linearis* were obtained during winter from several sites in Halifax County. Specimens from Martinique Beach in January were mature with thalli divided into male and female portions. Plants from Finck Cove in early February had only small numbers of mature spores, mostly with male portions but a few with female portions; specimens taken late in the month and early March were well matured, although bearing only male portions as were plants collected at Peggy Cove in April. Numerous nuclei in division and with chromosomes, both in male and female portions, were obtained in plants from Martinique Beach. The chromosome number was five in spermatangia (Pl. III, A-B) and ten in the divisions leading to carpospores (Pl. III, C-F). In formation of the carpospores, the first cell division occurred vertically through the long axis of the cell, although in some cases the plane of division was oblique (Pl. III, E-F). The chromosome number in spermatia from Finck Cove and Peggy Cove was also five. At prophase in spermatia of *P. umbilicalis*, two of the five chromosomes were longer, but this disparity in length was not noted in *P. linearis* even though numerous nuclei in prophase were observed.

4. *Porphyra* sp.

The thallus of this unnamed species is red to reddish-brown, umbilicate, 5 to 8 cm in diameter, monostromatic, and monoecious. When fertile these plants contain 128 spermatia and 16 carpospores in each packet. This alga is quite similar to *P. umbilicalis*, both in structure and in morphology. The distinguishing feature of *Porphyra* sp. is its epiphytic habit on *Ascophyllum nodusum*. I collected it at Martinique Beach, Halifax Co., in September. At that time *Porphyra* sp. was already fertile, and had disappeared by early January. Contrariwise, *P. umbilicalis* was infertile in September, and was found abundantly in January. This alga probably has been referred to as *P. umbilicalis* f. *epiphytica* COLLINS (TAYLOR, 1957), but since it has a different number of chromosomes than does *P. umbilicalis*, I consider it a separate, and at this time, an unnamed species.

The chromosome numbers of *Porphyra* sp. were n=3 (Pl. III, G-I) in formation of spermatia and 2n=6 (Pl. III, J-L) in formation of carpospores. This complement of chromosome is the same as in *P. leucosticta*, but in the present instance one of the chromosomes in the division leading to spermatium is longer than the other two (Pl. III, G-H).

Discussion

Chromosome numbers in species of *Por-*

phyra from Nova Scotia were: n=3, 2n=6 in *P. leucosticta* and *Porphyra* sp., and n =5, 2n=10 in *P. umbilicalis* and *P. linearis*.

KRISHNAMURTHY (1959) found that in *P. umbilicalis* var *laciniata* (=*P. purpurea*) neither fertilization nor reduction division occurred in the life history of this alga. In contrast the four species of *Porphyra* from Nova Scotia all had the haploid number of chromosomes in spermatia and the diploid number in carpospores.

My observations on both *P. umbilicalis* and *P. leucosticta* were imcoplete inasmuch as I was unable to elucidate the nuclear phases throughout the life history of these two species. However, in *P. umbilicalis* I have shown that the conchocelis was diploid as has been reported in *P. linearis* (GIRAUD and MAGNE, 1968), *P. miniata* (KITO *et al.*, 1971), *P. pseudolinearis* (KITO, 1974), *P. tenera* (KITO, 1974), and *P. yezoensis* (KITO, 1967, 1974; MIGITA, 1967). In addition dwarf plants of *P. leucosticta* were shown to be haploid.

My chromosome count for *P. linearis* does not agree with those presented by previous investigators (DANGEARD, 1927; KITO *et al.*, 1967; MAGNE, 1952; GIRAUD and MAGNE, 1968). Possibly the chromosome number in the same species varies spatially, but a more likely explanation is that several species are being reffered to as *P. linearis*, a situation analogous to that reported by COLL and COX (1970). It is evident that species delimitation in the genus *Porphyra* frequently is ambiguous, and certainly in the northwestern Atlantic further study is required.

This study was done at the National Research Council of Canada, Atlantic Regional Laboratory in Halifax, while I was a visiting scientist from September 1976 to July 1977. I am grateful to Dr. J. Mc LACHLAN for providing facilities and suggestions, and I am equally indebted to the late Dr. T. EDELSTEIN, Dr. L. C-M. CHEN, and Miss C. J. BIRD for their generous help and useful criticisms. Mr. M. GREENWELL, Mr. W. R. CROSBY, and Mr. J. R. VAN DER MEER also provided assistance for which I am thankful.

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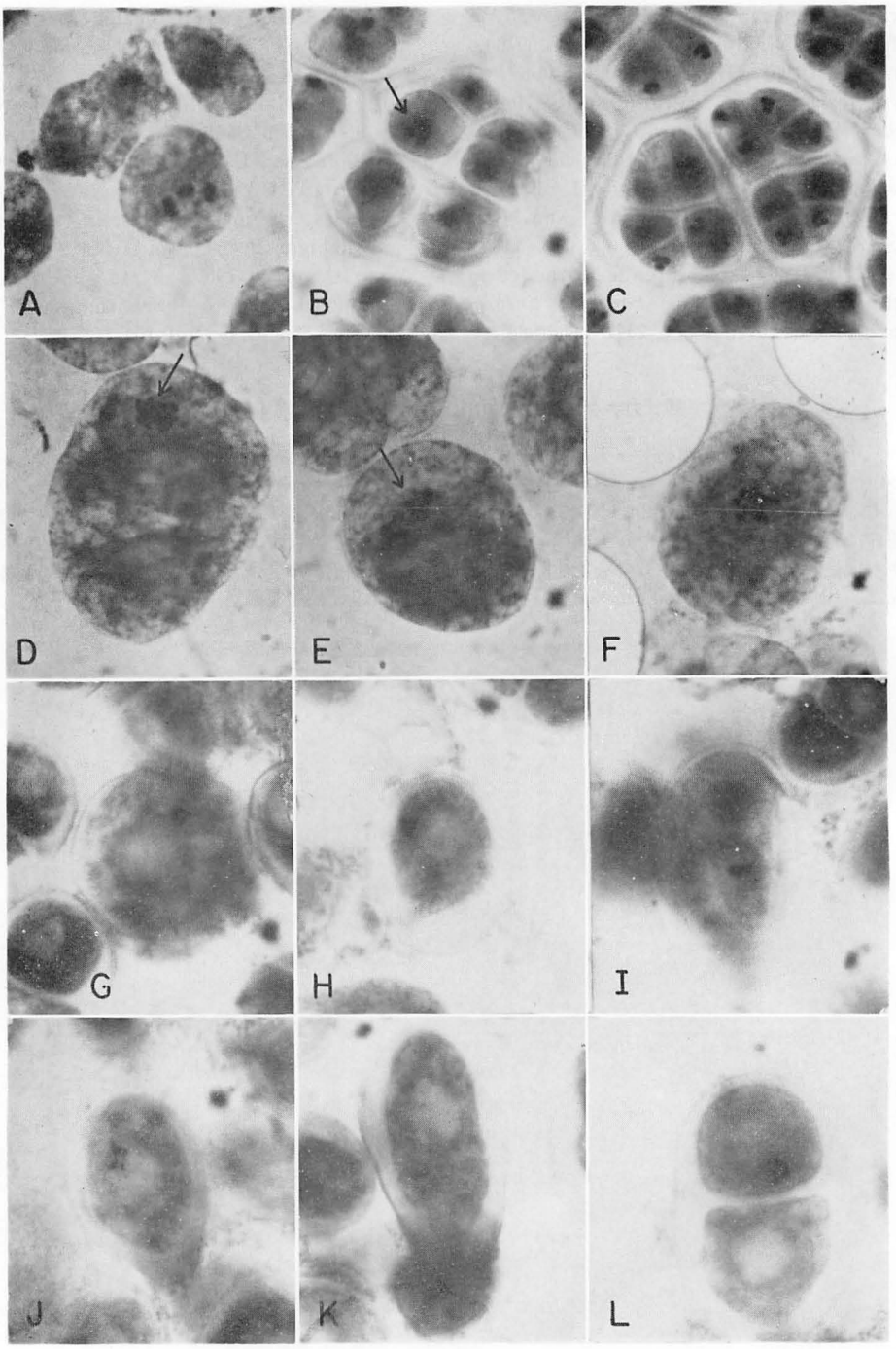
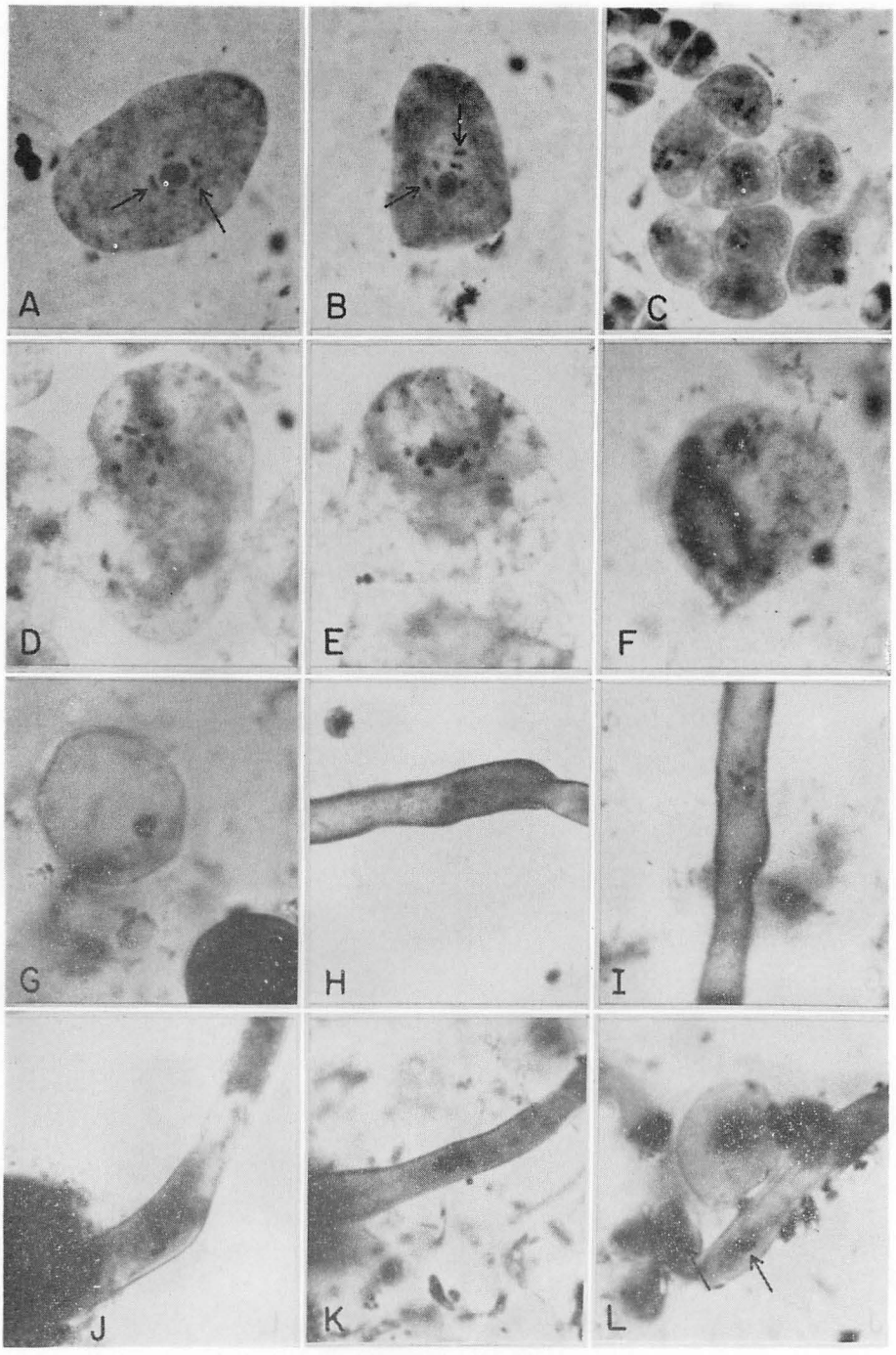
籔　淵：カナダ・ノバスコシア産アマノリ属植物4種の染色体数

カナダ，ノバスコシア産4種のアマノリ属植物，*Porphyra leucosticta*, *P. umbilicalis*, *P. linearis*, *Porphyra* sp. について細胞学的研究を行った。染色体数は *P. leucosticta* と *Porphyra* sp. では n=3, 2n=6, *P. umbilicalis* と *P. linearis* では n=5, 2n=10 であった。精子形成の際の核分裂前期には *Porphyra* sp. では1個の染色体が他の2個よりも長く，*P. umbilicalis* では2個の染色体が他の3個よりも長い。培養では *P. leucosticta* の胞子から発生した小葉状体は半数の染色体，*P. umbilicalis* の胞子から発生した糸状体は倍数の染色体を有することを見た。(040 函館市港町3丁目1-1，北海道大学水産学部水産植物学教室)

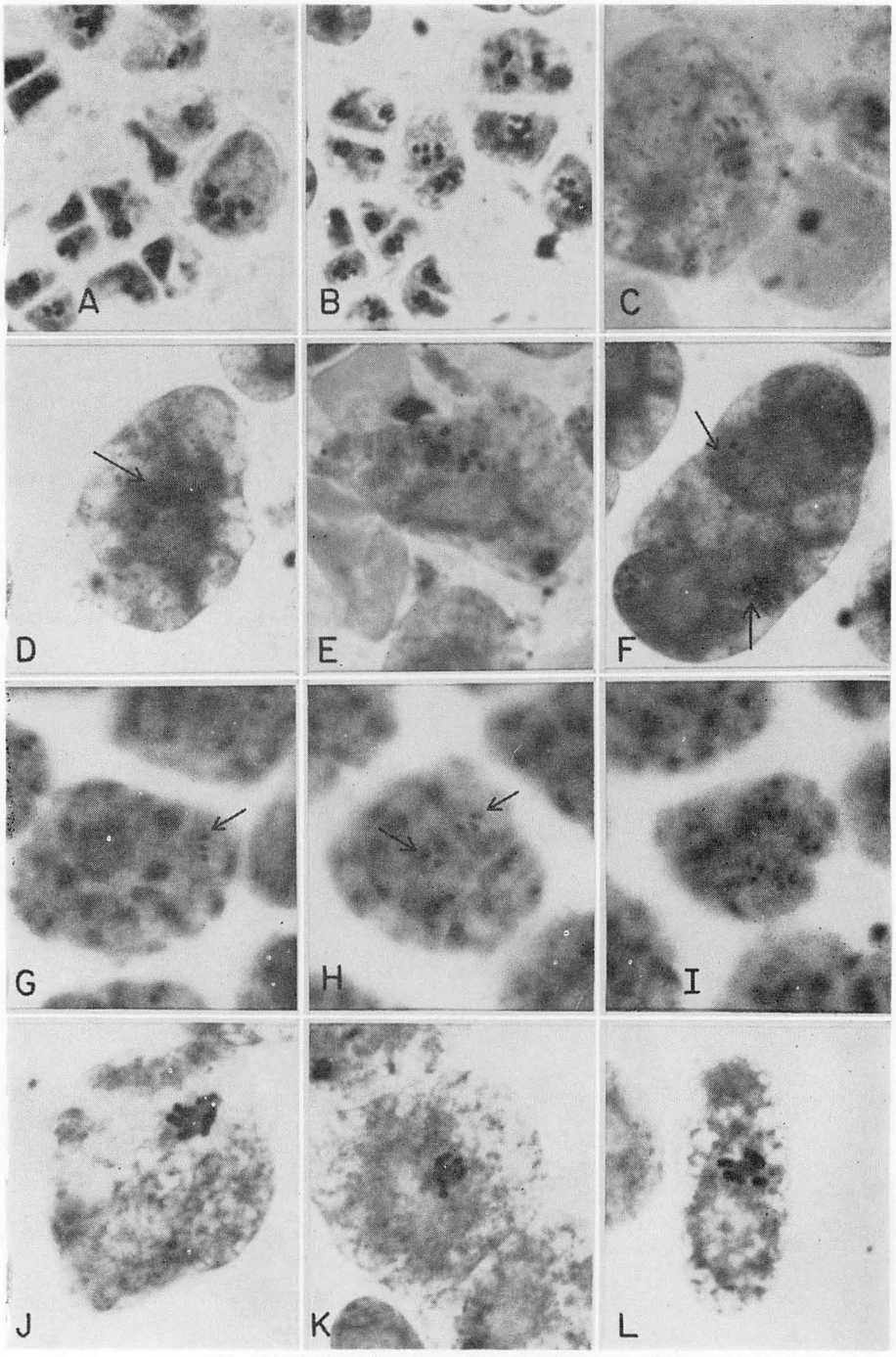


Pl. I. *Porphyara leucosticta* THUR. in LE JOL. ×800.
A–C. Chromosomes in various stages in cells leading to formation of spermatia. Arrow in B indicates three rugged chromosomes in metaphase. D–F. Late prophase (D) and early metaphase (E–F) in first division of diploid nucleus in formation of carpospores. Arrows in D and E indicate nuclei. G–L. Nuclear division leading to dwarf plant from spore of leafy thallus; G–H: metaphase of first division; J–K: anaphase of first division; L: metaphase in two-celled phase.



Pl. II. *Porphyra umbilicalis* (L.) J. Ag. ×800.

A-C. Chromosomes in various stages in cells leading to formation of spermatia. Arrows indicate long chromosomes which are not distinct from the others in B because of bending. D-E. Late prophase nucleus in first (D) and second (E) cell division in formation of carpospore. F-G. Prophase (F) and metaphase (G) in spores discharged from leafy thallus. One large nucleolus and small granules of chromatin are evident in F. H-L. Various stages of nuclear division in cells of conchocelis. H: prophase with two nucleoli; I-K: metaphase; L: late anaphase. Groups of daughter chromosomes are seen as curved line (arrow).



Pl. III, A-F. *Porphyra linearis* GREV. ×800.

A-B. Chromosomes in cells leading to formation of spermatia. C-D. Prophase (C) and early metaphase (D) in first division of diploid nucleus leading to formation of carpospore (arrow indicates nucleus). E-F. Early metaphase of diploid nucleus in two-celled stage leading to formation of carpospore (arrows in F indicate nuclei).

G-L. *Porphyra* sp. ×800.

G-I. Chromosome in cells leading to formation of spermatia (arrows indicate long chromosmes). J-L. Chromosomes in late prophase of diploid nucleus in formation of carpospores.