MORPHOLOGICAL AND DEVELOPMENTAL STUDIES OF GELIDIALES II. ON *ACANTHOPELTIS JAPONICA* OKAMURA

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Since the genus Acanthopeltis was established by Okamura in 1900, only one species, *A. japonica* has been known from the warmer parts of the Pacific coasts of Japan and from Korea. Okamura distinguished this alga from other members of the Gelidiales by its characteristic habits, the peculiar manner of growth by proliferation, and its sympodial branching which presents a remarkable contrast to the monopodial branching in other genera.

Structures of the thallus, the mature cystocarp and the tetrasporangium were described and illsutrated by Okamura (1900), and recently details of the development of cystocarp were studied by Fan (1961) with material from Japan. However, the male reproductive organ of this alga has remained unknown until today. It was fortunate enough to discover a number of individuals bearing antheridial ramuli together with female ones among the material from Shimoda, Shizuoka Prefecture. Cytological observations of the tetrasporgangium development were also carried out. In this paper are reproted the results of my studies and a discussion on the morphology of this alga.

I am greatly indebted to my teacher Dr. J. Tokida, Professor Emeritus, for his guidance and also for his kindness in reading the manuscript, and to Dr. T. Masaki, Dr. H. Yabu and Dr. Y. Saito of Hokkaido University for their kind advice and help in many ways. I also wish to acknowledge the gentlemen of the Izu Branch of Shizuoka Prefectural Fishery Experiment Station at Shimoda in collecting the material.

Material and Methods

The material of this study was collected at Shimoda, Shizuoka Prefecture, while I was staying at the Station from the 5th through the 30th of May, 1964. Most of the specimens collected were preserved in 10% formalin sea-water. Other specimens for cytological studies and for observation of the reproductive organs were fixed either with Tahara's fluid or with a mixture of absolute alcohol and acetic acid (3:1). The specimens fixed were stained with haematoxylin and aniline

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blue, while those fixed with the latter mixture were squashed and stained with iron-alum aceto-carmine (cf. Rao, 1953; Austin, 1959). For chromosome counting, the latter method gave more satisfactory results, though the sporangia in a squashed preparation were of a larger size than the natural one as a result of their exposure to pressure and heat. The nucleus, nucleolus and chromosomes were found to retain their natural size in such a preparation.

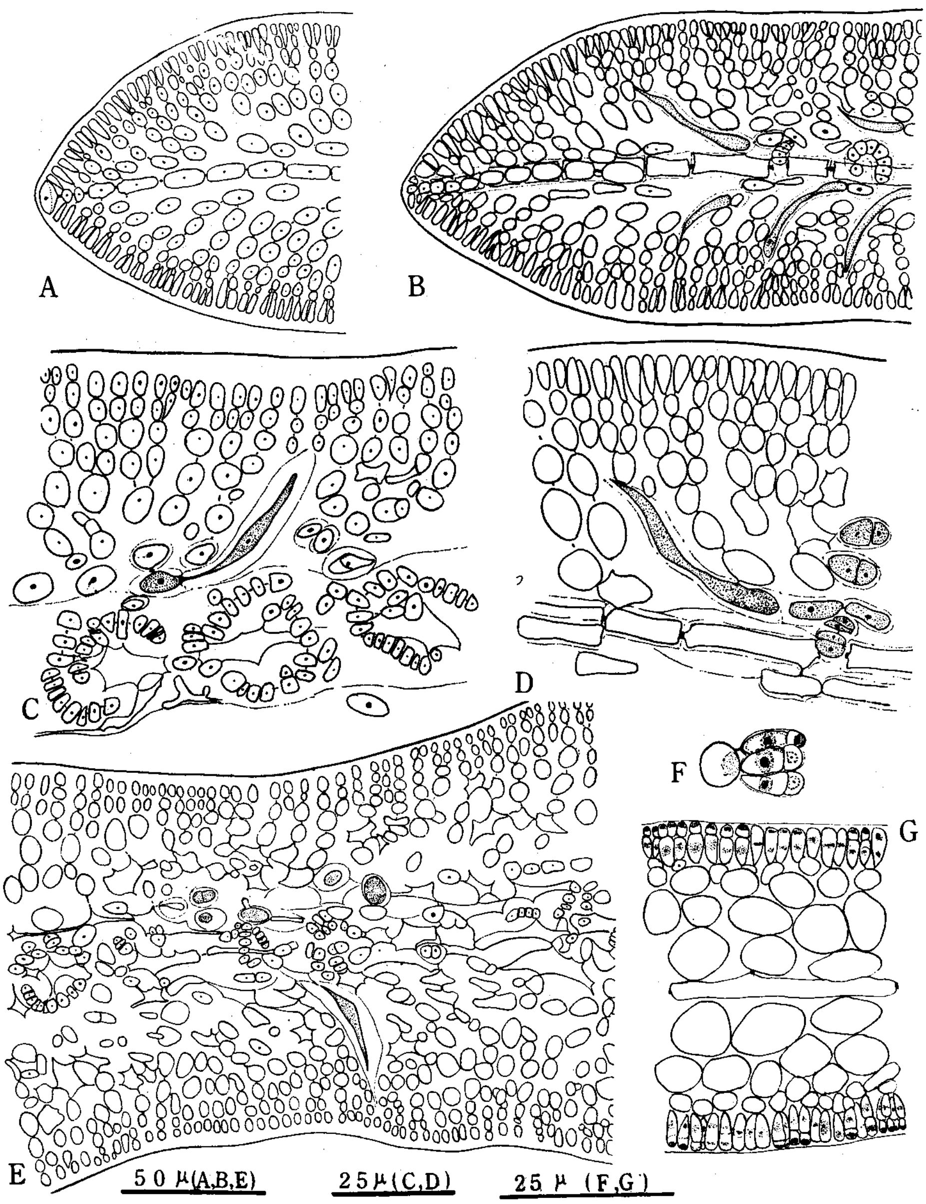
Results

1. Sexual plant and development of sexual organs

So far as I have examined, the present alga is monoecious. Among the specimens in my collection which amount to 67 in number, 28 are sexual and provided with both spermatangial and female ramuli, 29 are tetrasporic, and 10 are sterile. The sexual plant is not readily distinguishable from the tetrasporic with the naked eye, but it is possible with the help of a magnifying glass because the fan-shaped, flattened and thin (41–94μ thick) male ramuli are in a striking contrast to the subcylindrical and thick (130–172μ thick) tetrasporic ones.

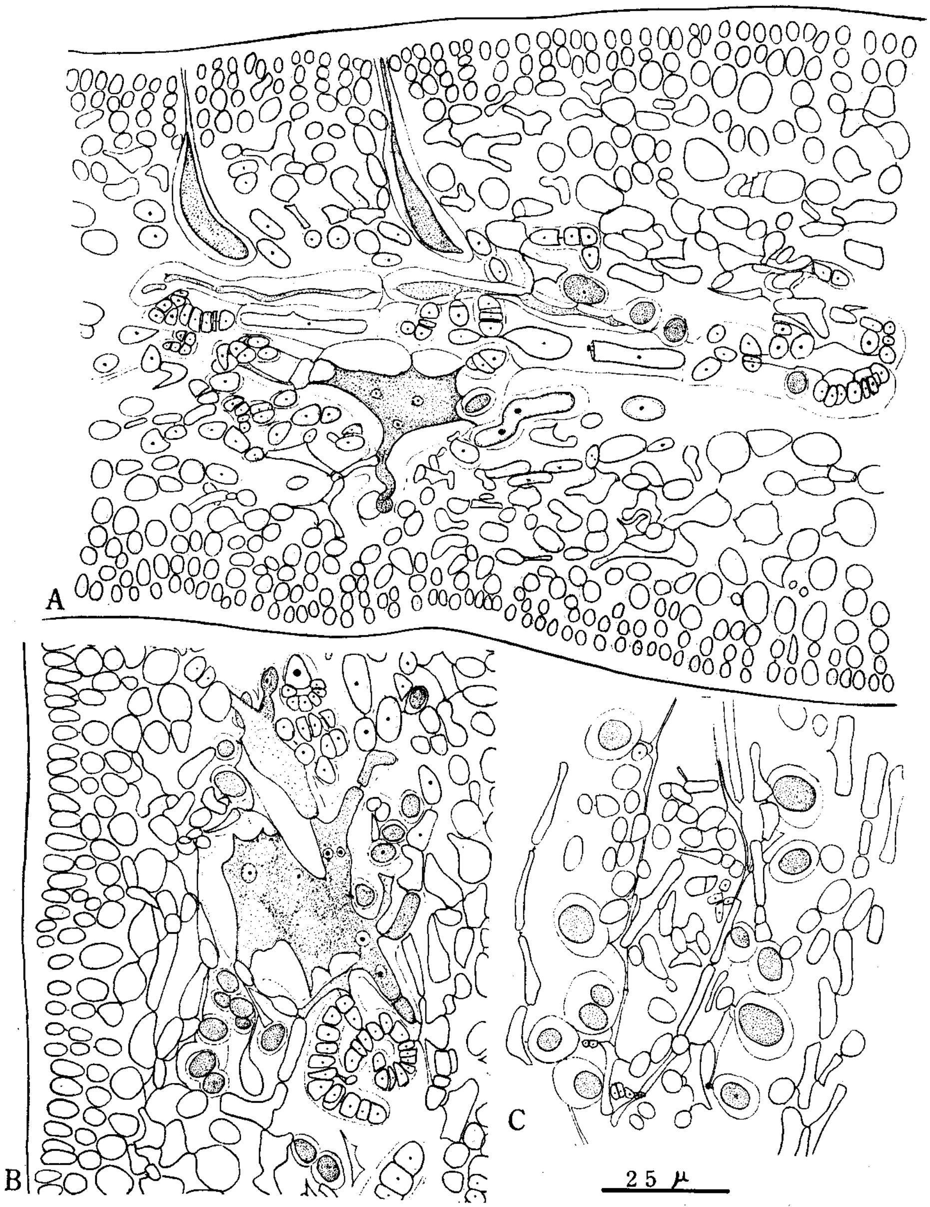
The spermatangial mother cells are formed in the same way as in other members of Gelidiales (cf. Kylin, 1928; Dixon, 1958; Fan, 1961). The superficial cells of both surfaces of the flattened fertile ramuli (Pl. II, Figs. 1 & 2) divide vertically to produce spermatangial mother cells, and the spermatangia are produced from the mother cells by their transverse divisions (Text-fig. 1, F & G; Pl. II, Fig. 5; Pl. III, Figs. 1 & 2). The spermatia are ellipsoidal in shape, $1.7\mu \times 3.4\mu$, almost colorless, and with a large nucleus in shape, in the anterior portion (Text-fig. 1, F; Pl. II, Fig. 2). The liberation of the spermatia is associated with the decay of the surface membrane.

The development of female reproductive organs in this alga was well illsutrated by Fan (1961, Text-fig. 9). They are formed in a special ramulus (Pl. II, Figs. 1 & 3). As shown in Pl. III, Figs. 3–4, the carpogonium is intercalary or lateral on the second basal cell of a branch of the third order developed on both sides of the central axis of the female ramulus. The carpogonial branch is always one-celled (Text-fig. 1, B-D). During the development of carpogonium, nutritive filaments of small cells are formed on every basal cell of branches of the third order in the fertile part (Text-fig. 1, C-E; Pl. III, Fig. 5). Cells of the nutritive filaments have dense cytoplasmic contents which stain well with haematoxylin (Pl. III, Fig. 5). After fertilization, the trichogyne degenerates (Text-fig. 1, E; Pl. III, Fig. 5; Pl. IV, Fig. 1) and the carpogonium fuses with the supporting cell and the neighbouring cells to form a large irregular fusion cell containing two to four nuclei (Text-fig. 2, A & B; Pl. IV, Figs. 2–4). The fusion cell gives rise to several processes toward the central axis cutting off gonimoblast initials (Text-figs. 2, B; Pl. IV, Figs. 2–4) which develop and divide to form gonimbolast filaments from

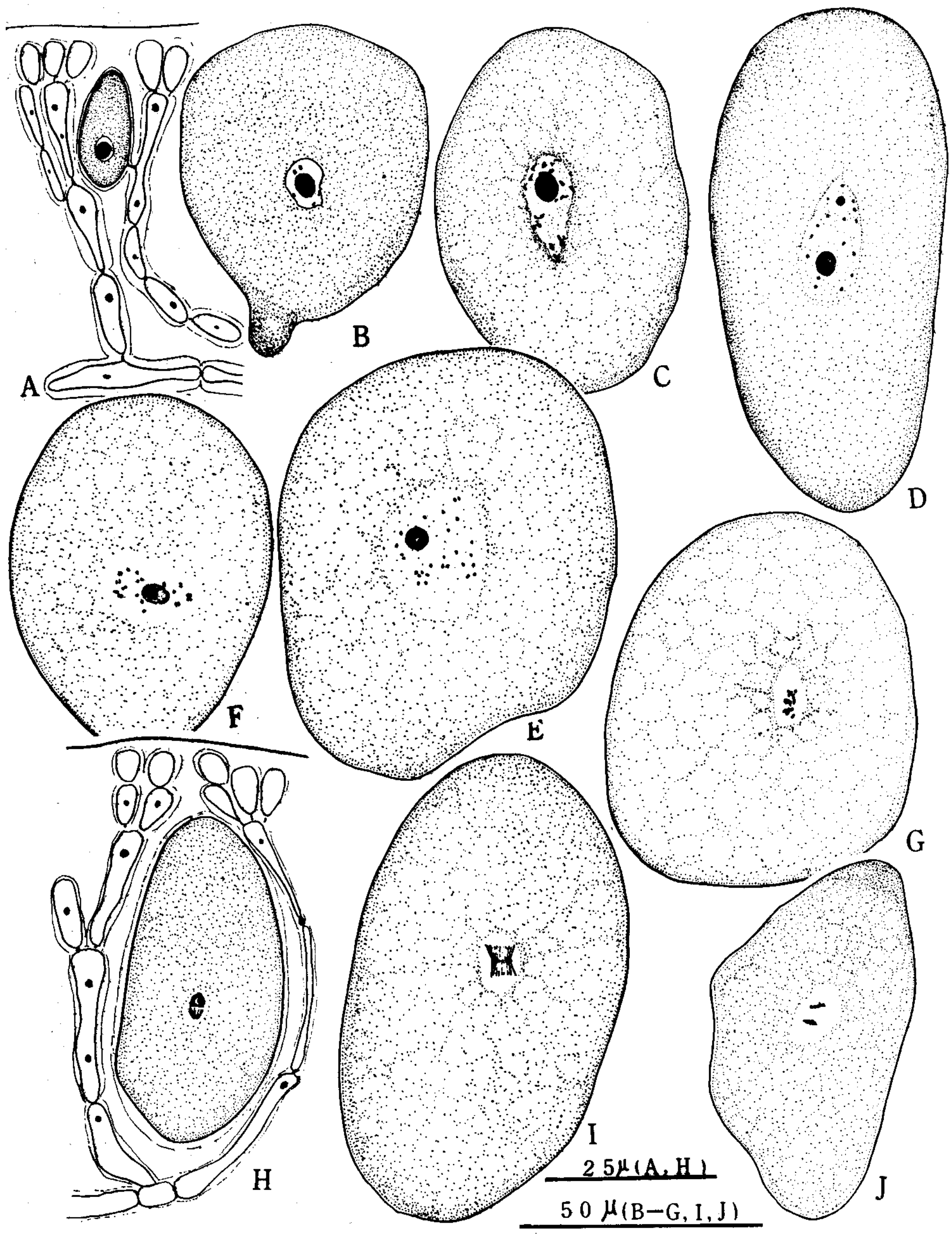


Text-fig. 1. *Acanthopeltis japonica* Okamura

A. Longitudinal section through the apical poritíon of a ramulus. B. Longitudinal section through a young female ramulus. C & D. Longitudinal section through a female ramulus showing a carpogonial branch and nutritive cells. E. Longitudinal section through a female ramulus after fertilization. F. Detail of spermatangia. G. Longitudinal median section through a spermatangial ramulus.



Text-fig. 2. *Acanthopeltis japonica* Okamura
A & B. Longitudinal section through a female ramulus showing a large irregularly-shaped fusion-cell. C. Part of medulla showing young carposporangia.



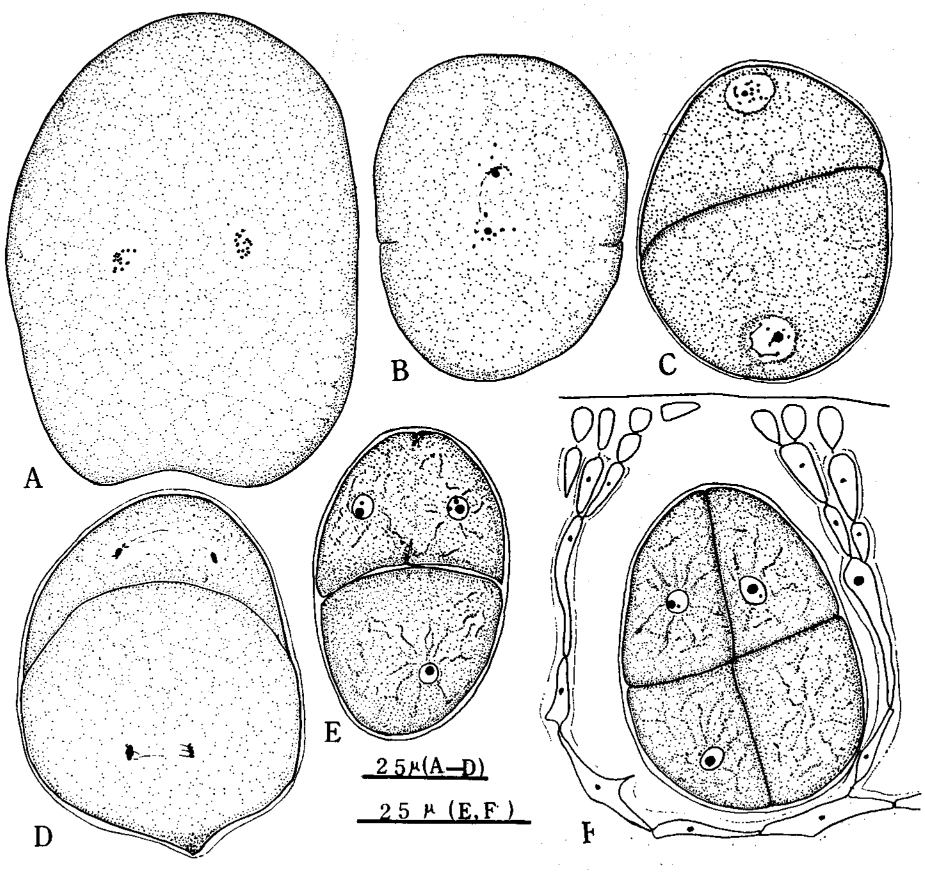
Ttext-fig. 3. *Acanthopeltis japonica* Okamura

A. Tetrasporangial initial. B-J. Young tetrasporangia showing various stages of nuclear division; B, resting stage; C & D, prophase; E, late prophase; F, diakinesis; G-I, metaphase; G, showing 15 bivalent chromosomes; H & I, showing spindle in side view; J. anaphase.

which the carposporangia are cut off toward the surfaces of the ramulus (Text-fig. 2, C). Unfortunately the specimens examined were so young that no further development of gonimoblasts could be observed. Okamura (1900) and Fan (1961) illsutrated the mature cystocarps of the present species as bilocular.

2. Development of tetrasporangia

Tetrasporngaia are formed from the cortical cells of the tetrasporic ramuli which are usually flat leaflets (Pl. II, Fig. 4). The tetrasporangial mother cell develops from a terminal cell of a lateral cell filament (Text-fig. 3. A; Pl. V. Fig. 2). The mother cell is uninucleate and 12–17$\mu\times$8.5–12μ in size (Text-fig. 3, A; Pl. V. Fig. 2). The nucleus is 3.5μ in diameter. The resting nucleus in a young



Text-fig. 4. *Acanthopeltis japonica* Okamura
A. Anaphase, showing 15 chromosomes in each group migrating toward the pole. B. Telophase, showing early stage of cell cleavage. C. Two-celled sporangium, each cell with a prophase of second nuclear division. D. Anaphase of second nuclear division. E. Early stage of second cell cleavage. F. Cruciately partite mature tetrasporangium.

sporangium contains a nucleolus, and rarely also one to two chromospherules-like granules besides the nucleolus (Text-fig. 3, B; Pl. V, Fig. 4). With the growth of the sporangium, the nucleus becomes larger in its cavity (Text-fig. 3, C & D). The granules soon become filamentous. The filaments do not stain well with aceto-carmine (Pl. V, Fig. 5). They gradually spread out within the nuclear cavity and soon become chromosomes (Text-fig. 3, D; Pl. V, 6). In the late prophase of nuclear division, the nuclear membrane disappears, and the nucleolus becomes larger and it does not stain uniformly but appears to be vacuolated (Text-fig. 3, E & F; Pl. V, Figs. 7 & 8). At this stage, the chromosomes are sometimes counted up to 30 (Text-fig. 3, E & F; Pl. V, Figs. 7 & 8). Soon the chromosomes become paired and the diakinesis stage sets in. In the metaphase, the nuceoli become obscure, 15 bivalent chromosomes are counted (Text-fig. 3, G-I; Pl. VI, Figs. 1 & 4), and we can occasionally observe spindles (Text-fig. 3, H & I; Pl. VI, Fig. 3). The centrosome-like body is rarely visible at the pole of the spindle (Pl. VI, Fig. 4). In the anaphase, each chromosome group migrating toward the pole contains 15 chromosomes (Text-fig. 3, J; Text-fig. 4, A; Pl. VI, Figs. 5 & 6). In late anaphase, the chromosomes are obscure while two nucleoli become visible (Text-fig. 4, B; Pl. VI, Figs. 7 & 8). Then the sporangium is divided by a wall into two daughter cells (Text-fig. 4, C; Pl. VI, Fig. 9). Soon after the first division finished, the second division takes place (Text-fig. 4, C-E). As a result of these two successive nuclear and cell divisions, a crucitately partite, ellipsoidal tetrasporangium, ca. $68\mu \times 32\mu$, is produced (Text-fig. 4, F). The resting nucleus in mature sporangia is sometimes observed to contain one or two nucleospherules besides the single nucleolus (Text-fig. 4, E & F).

Discussion

The present paper reports that the male organ was discovered for the first time in *Acanthopeltis japonica* collected in May and that the Gelidiaceae was first established to contain a monoecious species in *A. japonica*. A reason why Fan (1961) faled to discover the male organ in his material of *A. japonica* may be that his material was collected in July when the female ramuli were fully mature and bore ripe cystocarps. In my material collected in May the female ramuli were still very young while the male ones were fully mature. Okamura (1900) describes that the cystocarp of *A. japonica* becomes mature in later seasons, from August to October.

This paper presents also the first reprot on both the cytology of *A. japonica* and the meiosis in Gelididales. The chromosome numbers of the species in Gelidiaceae counted by previous investigators and by myself are listed in the following table.

Table 1. Chromosome number of the species in Gelidiaceae

Material	chromosome number	investigator
Gelidium corneum	n=5, 2n=10	Dixon, 1954
G. latifolium		
carposporophytes	n=5, 2n=10	Dixon, 1954
tetraspore germlings	n(?)= 18	Boillot, 1963
G. latifolium var. *luxurians*		
vegetative cells	n=or 2n=25−30	Magne, 1964
Acanthopeltitis japonica		
tetrasporangia	n=15, 2n=30	Kaneko, in the present paper

Considering from these data, it may be concluded that the basic chromosome number in Gelidiales is five.

Summary

Acanthopeltis japonica Okamura is reported herein to have both female and male ramuli on one and the same individual, or in other wards, this species is established to be monoecious. Nuclear divisions in the tetrasporangium of this alga were proved to be meiosis, and the chromosome numbers, n=15 and 2n=30, were counted.

Literature

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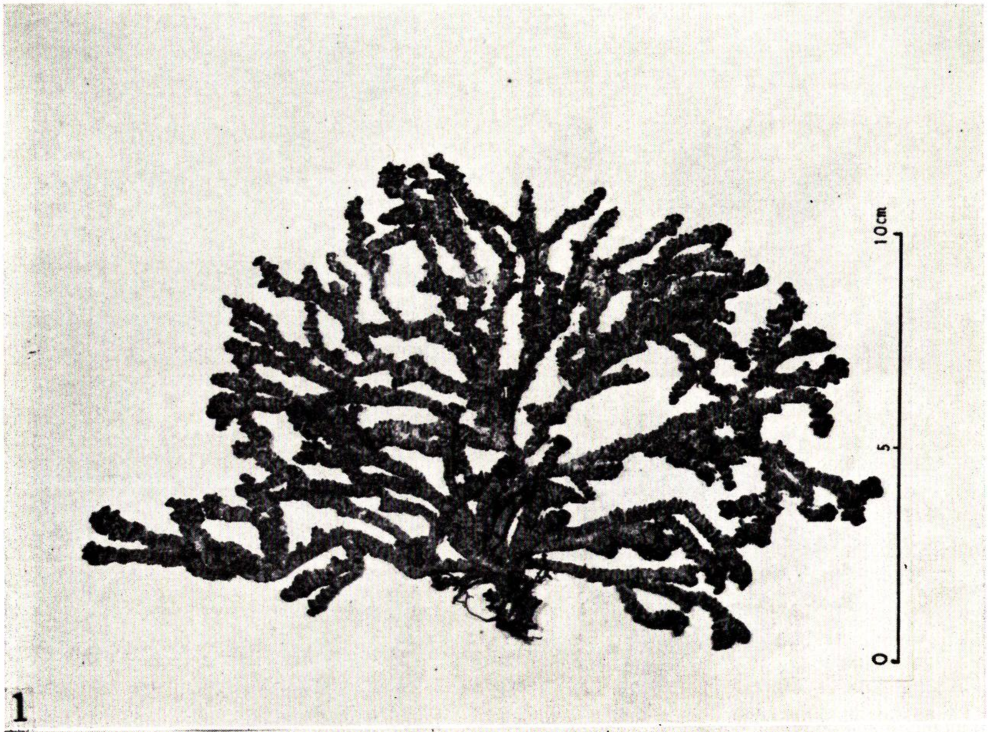
Explanation of Plates

PLATE I

Acanthopeltis japonica Okamura

Fig. 1. Habit of a sexual specimen
Fig. 2. Habit of a tetrasporangial specimen



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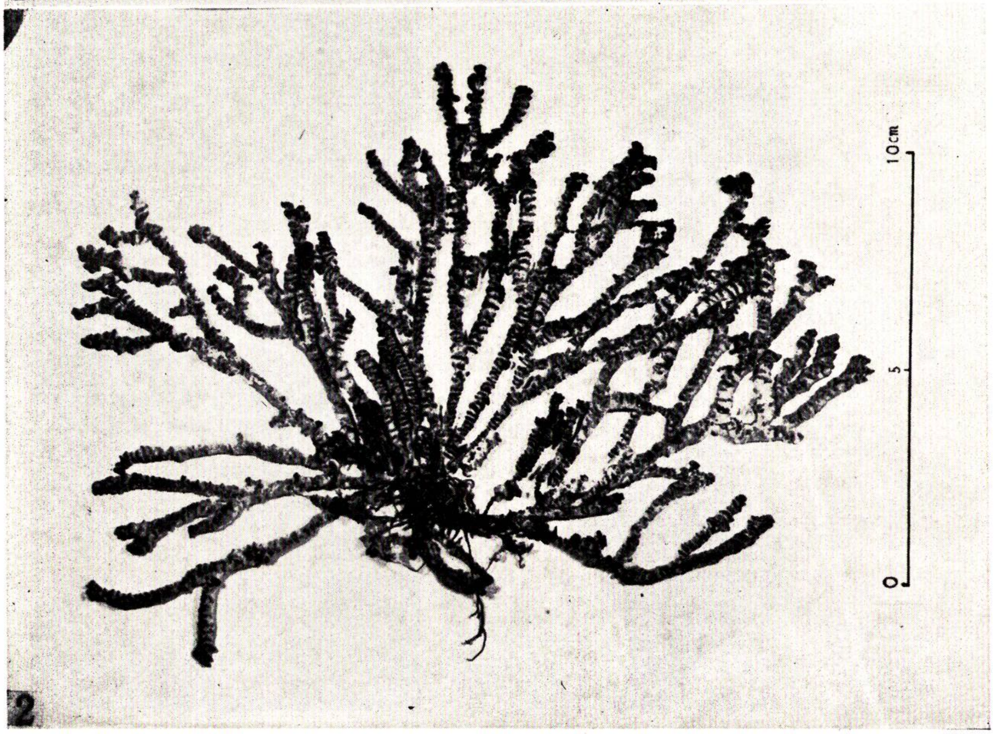

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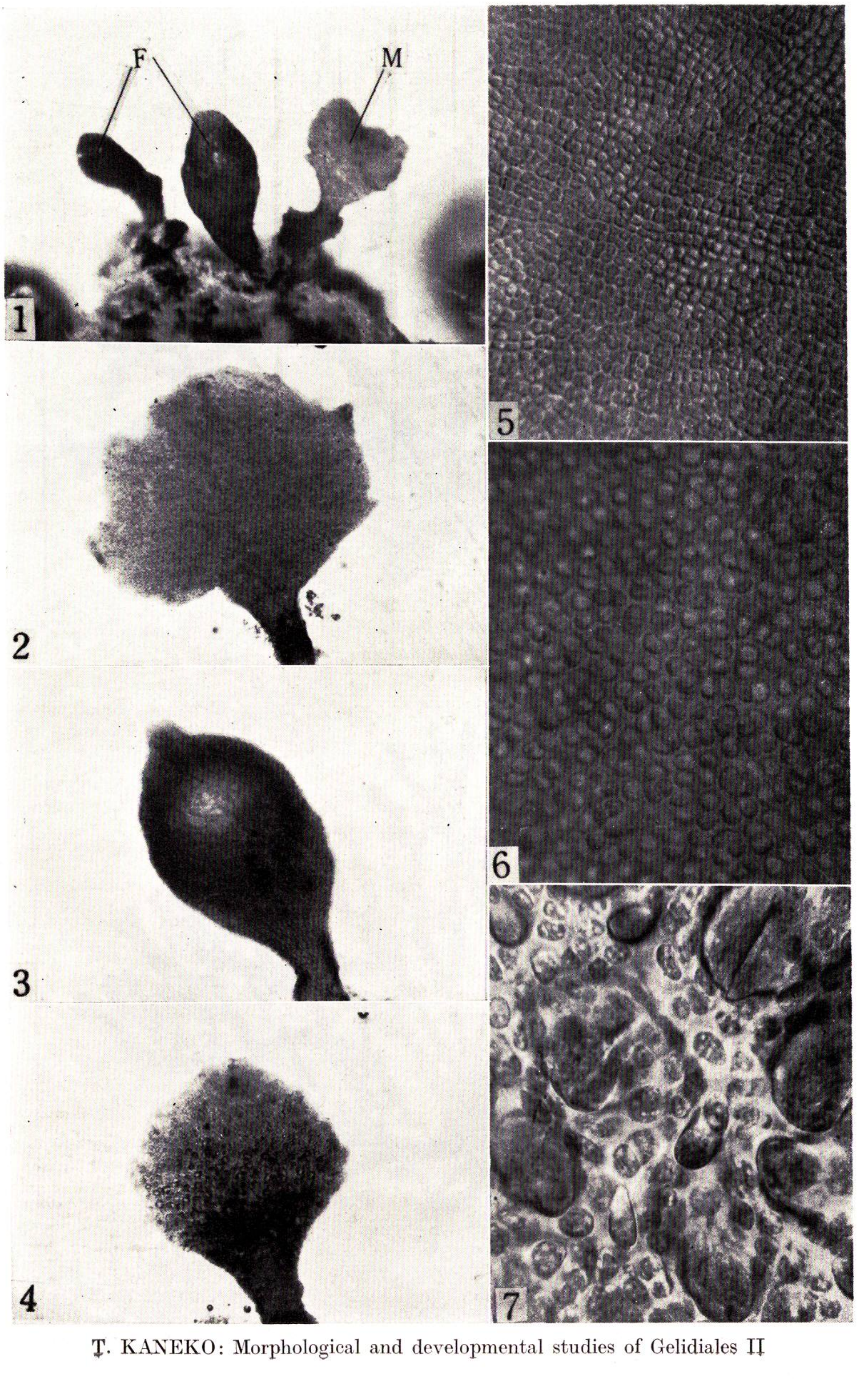

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PLATE II

Acanthopeltis japonica Okamura

Fig. 1. Part of a sexual frond showing a male and two female ramuli developed from the same frond ×14

Fig. 2. A male ramulus ×40

Fig. 3. A female ramulus ×40

Fig. 4. A tetrasporangial ramulus ×40

Fig. 5. Surface view of a male ramulus ×600

Fig. 6. Surface view of a female ramulus ×600

Fig. 7. Surface view of a tetraporangial ramulus ×600

PLATE III

Acanthopeltis japonica Okamura

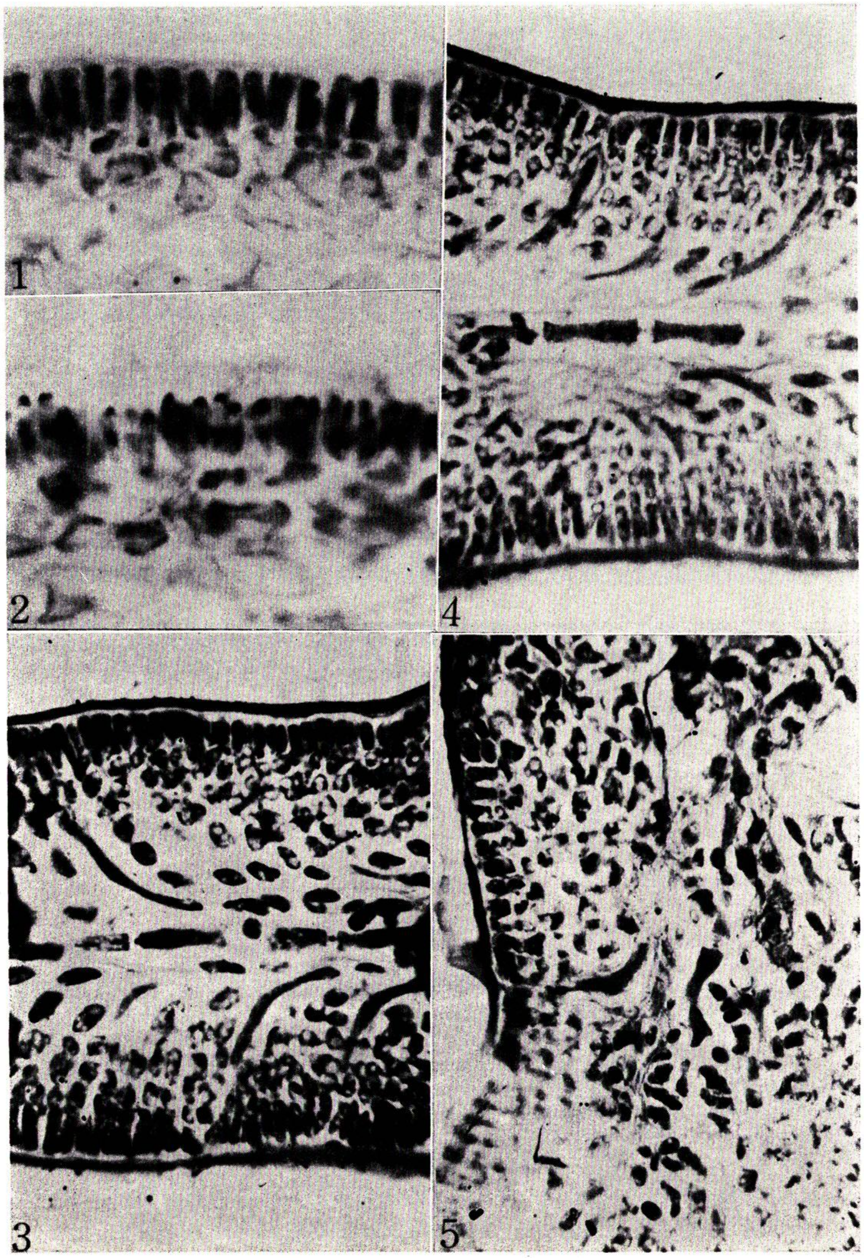
Fig. 1. Section through a young spermatangial sorus ×1440

Fig. 2. Section through a mature spermatangial sorus ×1440

Fig. 3. Longitudinal section through a mature female ramulus ×1300

Fig. 4. Longitudinal section through a mature female ramulus showing one fertilized carpogone and nutritive cells ×1300

Fig. 5. Longitudinal section through a female ramulus showing fertilized carpogonium and trichogyne ×1300



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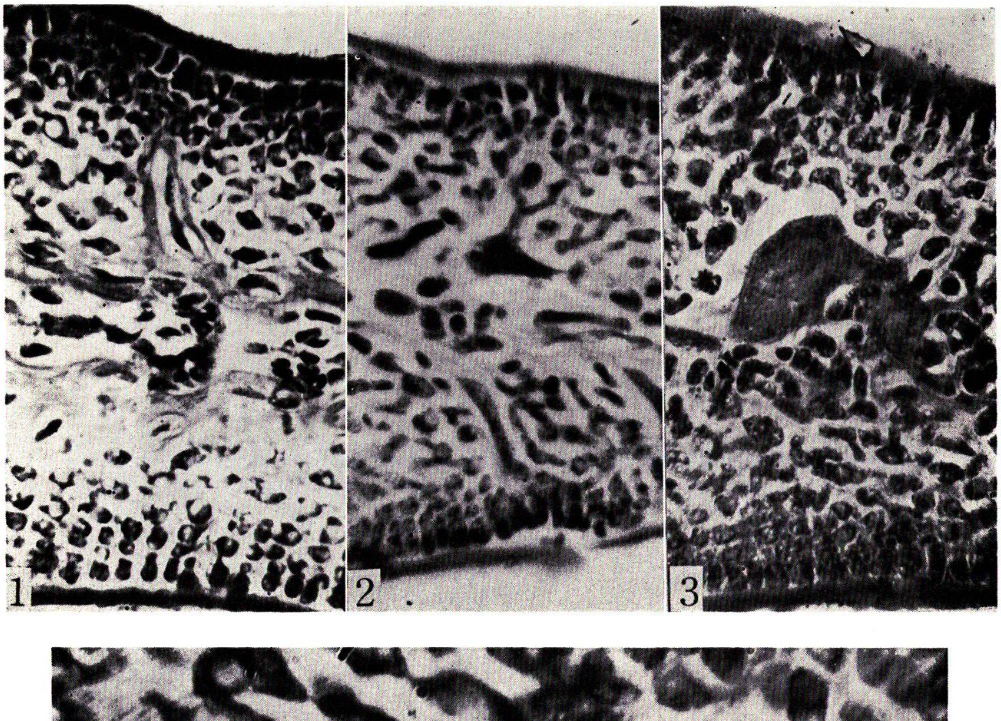

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PLATE IV

Acanthopeltis japonica Okamura

Fig. 1. Cross section through a female ramulus after fertilization ×1300

Figs. 2 & 3. Various stages after fertilization showing large fusion cells ×1300

Fig. 4. Part of large fusion cell showing protuberances and gonimoblast initials (FC, fusion cell; GON, gonimoblast initials; N, nucleus; NC, nutritive cells; P, protuberance) ×1440

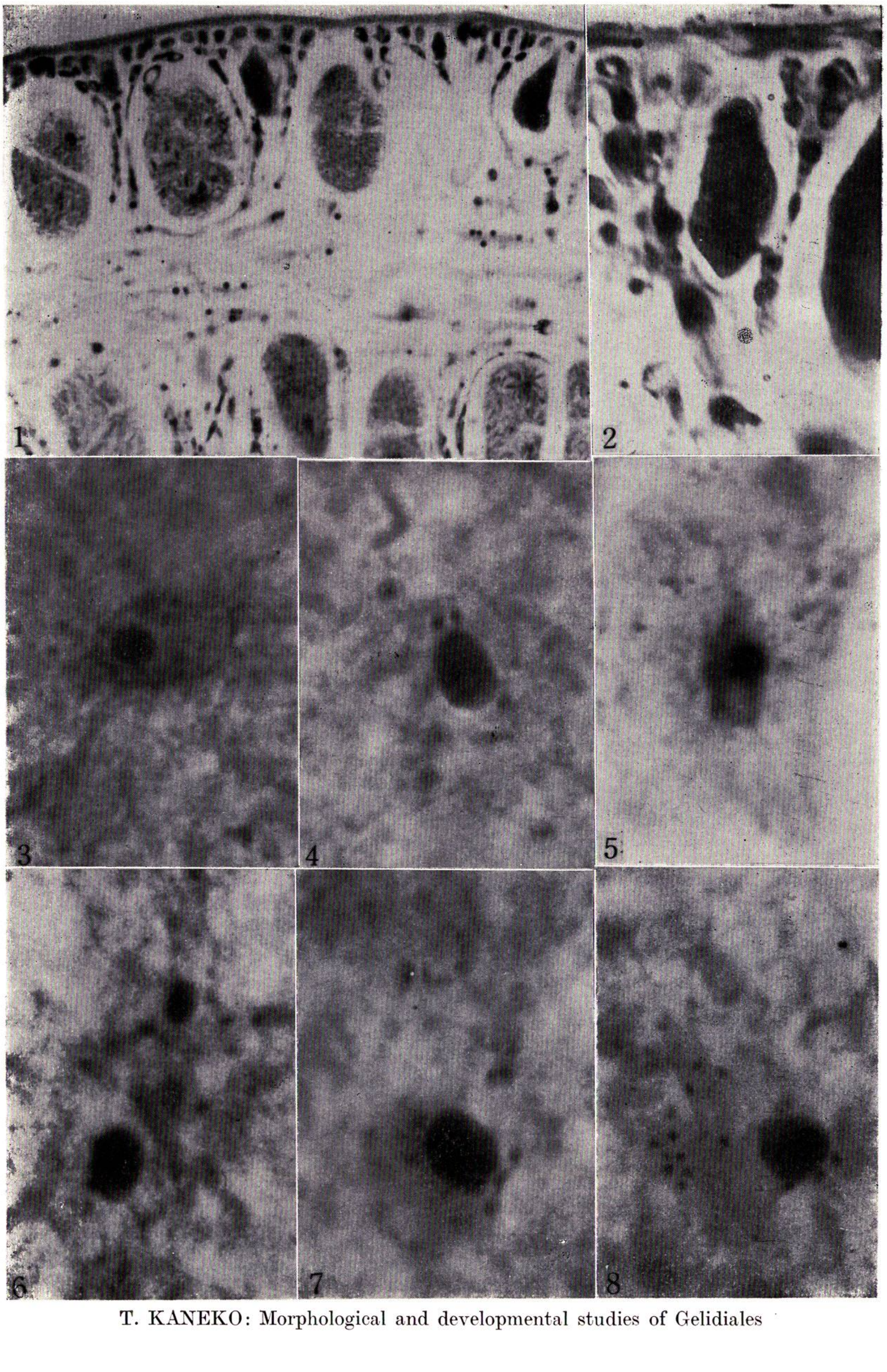
PLATE V

Acanthopeltis japonica Okamura

Fig. 1. Longitudinal section through a tetrasporangial ramulus $\times 290$

Fig. 2. Longitudinal section through a tetrasporangial ramulus showing young tetrasporangial mother cells $\times 1800$

Figs. 3–8. Various stages of nuclear divisions in tetrasporangia; Fig. 3. Resting nucelus showing nuclear membrane and one nucleolus; Fig. 4. Resting nucleus showing two chromospherule-like bodies besides one nucleolus; Fig. 5. Prophase of nuclear division, showing chromatin granules; Fig. 6. Late prophase showing several chromatin granules and two nucleoli; Fig. 7. Late prophase; Fig. 8. Diakinesis (Figs. 3–8, $\times 2036$) (Figs. 1, 2 & 5, stained with haematoxylin, and the others, stained with aceto-carmine)



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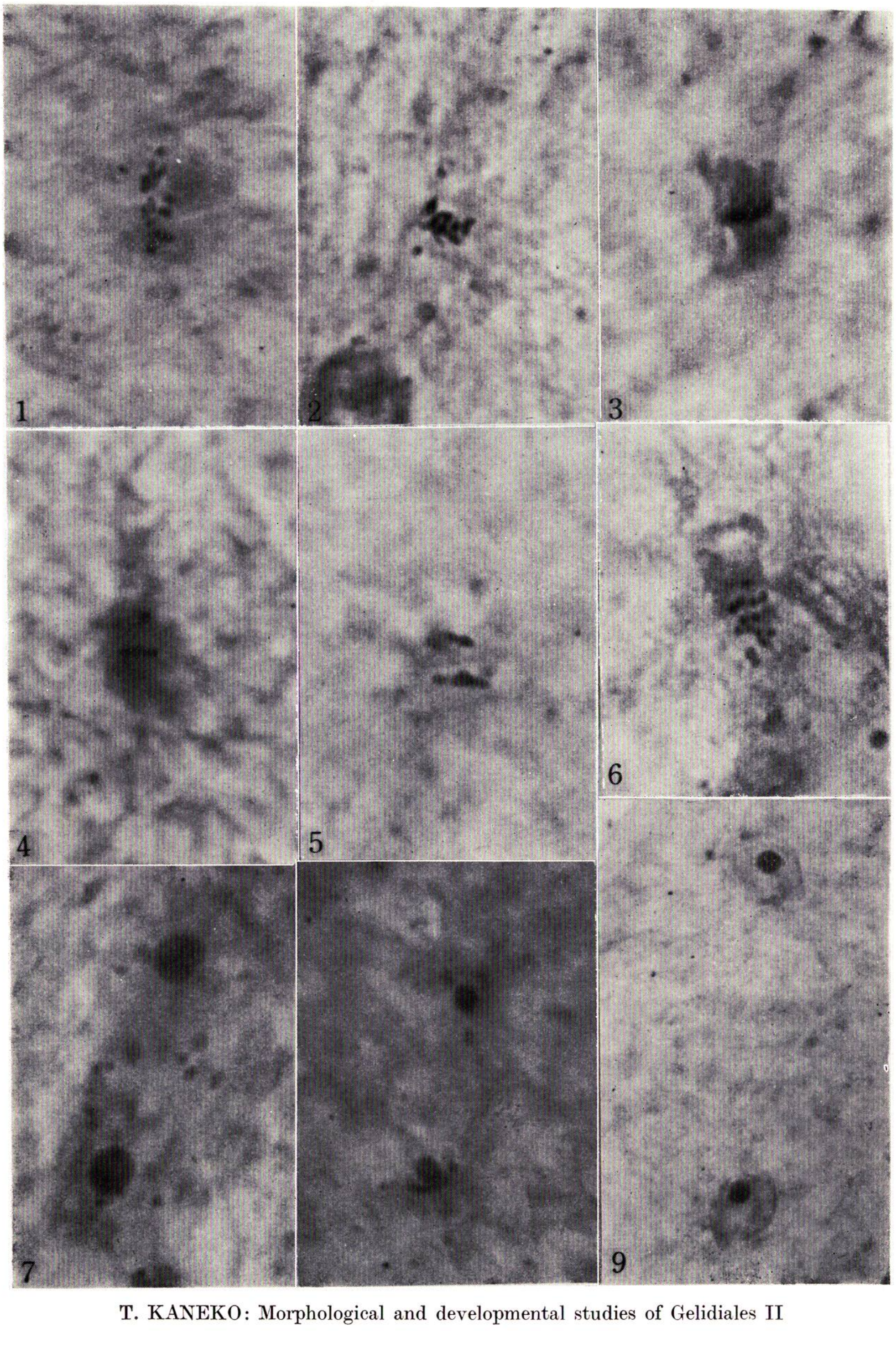

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PLATE VI

Acanthopeltis japonica Okamura

Figs. 1–4. Metaphase; Fig. 3. Side view of metaphase showing spindle; Fig. 4. Side view of metaphase showing a centrosome-like body

Figs. 5–8. Anaphase; Fig. 6. Part of anaphase showing two groups of chromosomes; Figs. 7 & 8. Late anaphase showing two daughter nuclei

Fig. 9. Telophase showing two daughter nuclei

(Figs. 1–9, ×2036; All stained with aceto-carmine)