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# Cytological observations on *Ptilota pectinata* (GUNN.) KJELLM. and *Pt. pectinata* f. *litoralis* KJELLM. (Ceramiales, Rhodophyta)

Hiroshi YABU

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Two species of *Ptilota*, viz., *Pt. pectinata* KJELLM. and *Pt. pectinata* f. *litoralis* collected from Hakodate and Usujiri, Hokkaido, have been studied cytologically. The first meiotic division in the tetrasporangium showed to have chromosomes of n=ca 34 in *Pt. pectinata* and n=ca 32 in *Pt. pectinata* f. *litoralis*. Among those chromosomes, the largest in mid-diakinesis comes to take O-shape in *Pt. pectinata* and 8-shape in *Pt. pectinata* f. *litoralis*. On the basis of the chromosome count in the cells leading to the formation of spermatium and carpospore, together with in the germlings from tetraspore and carpospore, *Ptilota pectinata* f. *litoralis* was indicated to be haploid in gametophyte and diploid in tetrasporophyte.

*Hiroshi Yabu, Faculty of Fisheries, Hokkaido University, Hakodate, 040 Japan.*

The red algae, *Ptilota pectinata* (GUNN.) KJELLM. and its forma, *Pt. pectinata* f. *litoralis* KJELLM., which have been reported to occur along the coast of Hokkaido (OKAMURA 1907; YAMADA & TANAKA 1944; TOKIDA & MASAKI 1959) are very common during spring and summer at Hakodate and its neighbouring Usujiri where the materials had been collected. So far as I am aware, the cytological informations have been lacking for the *Ptilota* group (KYLIN 1956), so the present study was undertaken.

## Materials and Method

The materials had been collected from May to July in the five consecutive years from 1971 to 1975 at two sites, Hakodate and Usujiri, in Oshima Province, Hokkaido. Several times, tetraspores and carpospores of both species had been discharged and cultured in SCHREIBER's solution to see the mitosis in their germlings. The favourable dividing nuclei were obtained only in the germlings of the spores from the thalli of *Pt. pectinata* f. *litoralis* which were collected at Tachimachi-misaki in Hakodate on May 29, 1974. The materials of the thalli and the spore germlings were both fixed in 3 parts absolute alcohol and 1 part glacial acetic acid and stained with WITTMANN's technique (WITTMANN 1965).

## Results and Discussion

*Meiosis in the tetrasporangium of* Ptilota pectinata *and* Pt. pectinata *f.* litoralis: The process of the meiotic division in the tetrasporangium of *Ptilota pectinata* and *Pt. pectinata* f. *litoralis* is quite identical, and it was very similar to that of *Chondria tenuissima* recently described by TÖZÜN (1976). Sometimes, however, the phase just like the so-called 'resting' or 'diffuse' stage which had been reported in *Palmaria palmata* (WESTBROOK 1928; MAGNE 1964; YABU, 1971) or *Gracilaria foliifera* and *Gracilaria* sp. (MCLACHLAN, et al. 1977), but not so conspicuous, was visible before diakinesis.

The chromosomes in early diakinesis in prophase I, come in sight as the tangled thin threads with several minute knots (Fig. 1) and they soon change their form

Table 1. Chromosome number counted at diakinesis and early metaphase from the nuclei with particularly good chromosome definition in the first tetrasporogenesis of *Ptilota pectinata* and *Pt. pectinata* f. *litoralis*

| Chromosome number | | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of nuclei | *Pt. pectinata* | 2 | 2 | 5 | 6 | 6 | 8 | 6 | 7 | 8 | 12 | 2 |
| | *Pt. pectinata* f. *litoralis* | 1 | 2 | 2 | 4 | 3 | 7 | 9 | 16 | 3 | 1 | |

to the distinct outline in small but various size (Figs. 2–5, 15–17).

The data of the chromosome count in diakinesis and early metaphase for two species (Table 1) showed the interesting feature indicating that the chromosome number differed between the species and it was n=ca 34 for *Pt. pectinata* and n=ca 32 for *Pt. pectinata* f. *litoralis*. The largest chromosome at mid-diakinesis takes O-shape in *Pt. pectinata* (Figs. 2–4) and 8-shape in *Pt. pectinata* f. *litoralis* (Figs. 16–17) and this was well discriminated at mid-metaphase as an extra large chromosome (Fig. 5). At anaphase II, this chromosome of *Pt. pectinata* represents the figure of X, of which one arm is somewhat shorter than the other one (Fig. 11). In anaphase I & II, two long trails were seen to be left behind the chromosome alignment, however, they were not so evident in *Pt. pectinata* f. *litoralis* (Figs. 20–21) as in *Pt. pectinata* (Fig. 10). Abberant side views of metaphase having odd chromosomes were encountered in considerably high frequency in *Pt. pectinata* (Figs. 6–9), whereas such views in *Pt. pectinata* f. *litoralis* (Figs. 20–21) were relatively a few. Rarely in *Pt. pectinata* f. *litoralis* one lagging chromatid (Fig. 21) was found at anaphase I & II. In both species the chromosome which was moving precociously toward each pole was occasionally seen in one of the daughter nuclei at metaphase II (Figs. 12, 22), and this was visible clearly to be separated from the chromosome group even in more advanced stages of later anaphase or early telophase, too (Figs. 13–14). From the view of this characteristic feature of its movement, I consider this chromosome to have close relation to the sex determination.

*Mitosis in the cell leading to the formation of spermatium and carpospore of* Ptilota pectinata *f.* litoralis: The dividing nuclei in male plants were easily obtained in the materials collected at any time in the cells leading to spermatium formation (Figs. 23–24), and the good figures usually showed 20–30 chromosomes. In female plants, the dividing nuclei were obtained not so easily as in males; they were found only in the materials collected in the middle of May, 1973 at Tachimachi-misaki. In those materials mature procarp and that with the spermatium nucleus through trichogyne (Fig. 25) had met with frequently. Nevertheless, I failed to observe the fact of the fertilization of male and female nuclei within the carpogonium. In the fused cell together with carpogonium and auxiliary cell and in the cells of the gonimoblast arising from the fused cell, 40–60 chromosomes were seen occasionally (Figs. 26–27).

*Mitosis in the spore germling of* Ptilota pectinata *f.* litoralis: The tetraspore and carpospore of *Ptilota pectinata* f. *litoralis* are nearly the same size of ca 36 μm in diameter which in somewhat smaller than those of *Pt. pectinata* (average ca 40 μm). In the same way as was described and figured by INOH (1947, p. 187–189) on the spore germlings of *Pt. pectinata*, tetraspore and carpospore from *Pt. pectinata* f. *litoralis* in culture had developed within two weeks after liberation into the thalli consisting of several seriated cells with one or several long rhizoidal filaments at its base (Fig. 36). When prophase sets in within one-cell stage of the development the nucleus increase its size rapidly until ca 28 μm in diameter, where

the numerous small chromatin granules appear within the nuclear cavity (Fig. 28). Soon the thin chromatin threads come to occupy the whole area of the nucleus. At late prophase in one or two-cell stage, I saw ca 30 chromosomes in tetraspore germlings (Figs. 29, 31–32) and 40–60 chromosomes in carpospore germlings (Fig. 36). In the tetraspore germlings, two chromosomes were somewhat longer than the others (Figs. 29, 31) and at anaphase they usually left a little longer trails behind the groups of chromosomes. As in the case of anaphase in the tetrasporogenesis, tetraspore germling was rarely seen to have one lagging chromatid still remaining near the equatorial position at late anaphase (Fig. 33). When each cell was completely formed by the acomplishment of the cell wall, the newly produced nucleus was found for a while connecting each other through pit connection of the cell (Fig. 34).

HANIC states (1973, p. 28) in the spore germling of *Chondrus* that "—at the four-cell stage; each cell may contain 2–4 nuclei." I (YABU 1976) saw in *Palmaria palmata* and *Rhodymenia pertusa* that the cell of the spore germling is essentially uninucleate, however some cells occasionally come to have multinuclei. In *P. pectinata* f. *litoralis* the germlings of tetraspore and carpospore, both have also uninucleate cells, however very rarely 2–4 nuclei were observed in only one-cell stage of the development. Unfortunately I was unable to ascertain whether each of these nuclei were effective for the later cell formation or the other nuclei except one became extinct in a little while. With the staining of Fe-propionocarmine after Feulgen, HANIC (1973) detected the conspicuous polar body at each end of the spindles in germling spores of *Chondrus crispus*. I tried this double staining for the tetraspore germlings of *P. pectinata* f. *litoralis*, but such a body could not be noticed in any of the germlings.

The chromosome counts in the spore germlings together with those in the formation of reproductive organs in tetrasporophyte and gametophyte described above confirmed the fact that in *Pt. pectinata* f. *litoralis* tetrasporophyte is diploid and gametophyte is haploid as has been known in many species of Rhodophyceae since YAMANOUCHI demonstrated on *Polysiphonia violacea* (=*P. flexicaulis*) in 1906.

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**簸　潡：クシベニヒバとコバノクシベニヒバについての細胞学的研究**

北海道の函館と臼尻に産する紅藻クシベニヒバとコバノクシベニヒバについて細胞学的研究を行った。四分胞子嚢内に於ける減数第一分裂でクシベニヒバではn＝約34，コバノクシベニヒバではn＝約32の染色体が数えられた。このうち最も大きい染色体はディアキネシス期にクシベニヒバではOの字型，コバノクシベニヒバでは8の字型を呈する。コバノクシベニヒバでは精子と果胞子形成過程の細胞並びに胞子発芽体で見た染色体数から配偶体は単相，四分胞子体は複相であることを確かめた。(北海道大学水産学部　040 函館市港町3丁目1-1)

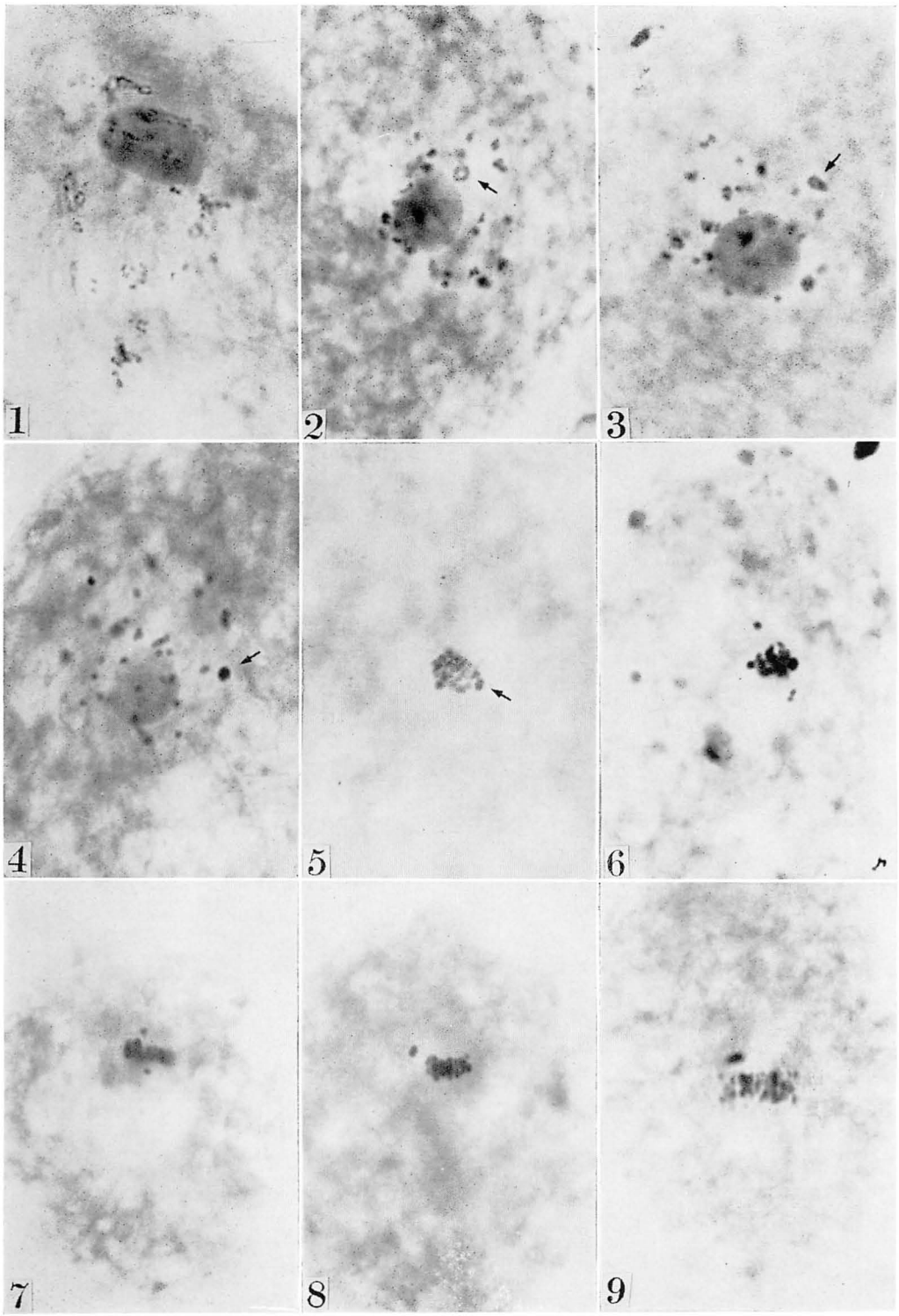
## Explanation of Plates

Figs. 1–9. Various stages of nuclear divisions in the tetrasporangia of *Ptilota pectinata* (GUNN.) KJELLM. All × 1,300. 1. Early diakinesis. 2–4. Mid-diakinesis. The largest chromosome taking O-shape is indicated by arrow. 5. Metaphase. Arrow indicates the extra large chromosome which was transformed from the O-shaped chromosome appeared at diakinesis. 6–9. Side views with one or two odd chromosomes at metaphase (Figs. 6–7) and early anaphase (Figs. 8–9).

Figs. 10–14. Various stages of nuclear divisions in the tetrasporangia of *Ptilota pectinata* (GUNN.) KJELLM. All × 1,300. 10. Anaphase. 11. Metaphase in the second division. 12. Side view of metaphase in the second division. 13–14. Anaphase in the second division. Arrow indicates the chromosome which behaves alone to be separated from the chromosome group.

Figs. 15–27. Various stages of nuclear divisions in the tetrasporangia of *Ptilota pectinata* f. *litoralis* KJELLM. Figs. 15–24 & 26–27, × 1,300; Fig. 25 × 130. 15–17. Diakinesis. Arrow in Figs. 16–17 indicates the largest chromosome taking characteristic 8-shape. 18–19. Side views of metaphase with odd chromosomes. 20. Anaphase in the second division. Arrow indicates one of the faintly stained a little longer trails leaving behind the chromosome alignment. 21. Anaphase in the second division. Arrow indicated one lagging chromatid. 22. Metaphase in the second division. 23. Late prophase (pointed by arrow) in the cell leading to spermatium formation. 24. Liberated spermatium with chromosomes (pointed by arrow). 25. Mature procarp with male nucleus (pointed by arrow) through trichogyne. 26. Late prophase of diploid nucleus in the fused cell of carpogonium and auxiliary cell. 27. Interphase or prophase nucleus in the cell of gonimoblast, Chromosomes are seen in the uppermost and lowermost cell in the figure.

Figs. 28–36. Various nuclear divisions in the spore germlings of *Ptilota pectinata* f. *litoralis* KJELLM. Figs. 28–34 & 36, × 1,300; Fig. 35, × 130. 28. Prophase in one-cell stage of tetraspore germling. 29. Late prophase in one-cell stage of tetraspore germling. Cleavage furrow toward the cell division already makes appearence faintly in the horizontal plane. Two long chromosomes are indicated by arrows. 30. Anaphase in one-cell stage of tetraspore germling. The trail leaving from the chromosome alignment is indicated by arrow. 31–32. Late prophase in two-cell stage of tetraspore germlings. In Fig. 31, the long chromosome is indicated by arrow. 33. Late anaphase in two-cell stage of tetraspore germling. One chromatid (pointed by arrow) is remaining still near the equatorial position. 34. Interphase nucleus in three-cell stage of tetraspore germling. The nucleus in each cell is elongated and is connecting through pit connection of the cell. 35. More advanced-stage than Fig. 34. 36. Metaphase in one-cell stage of carpospore germling. Cleavage furrow toward the cell division makes appearence already faintly.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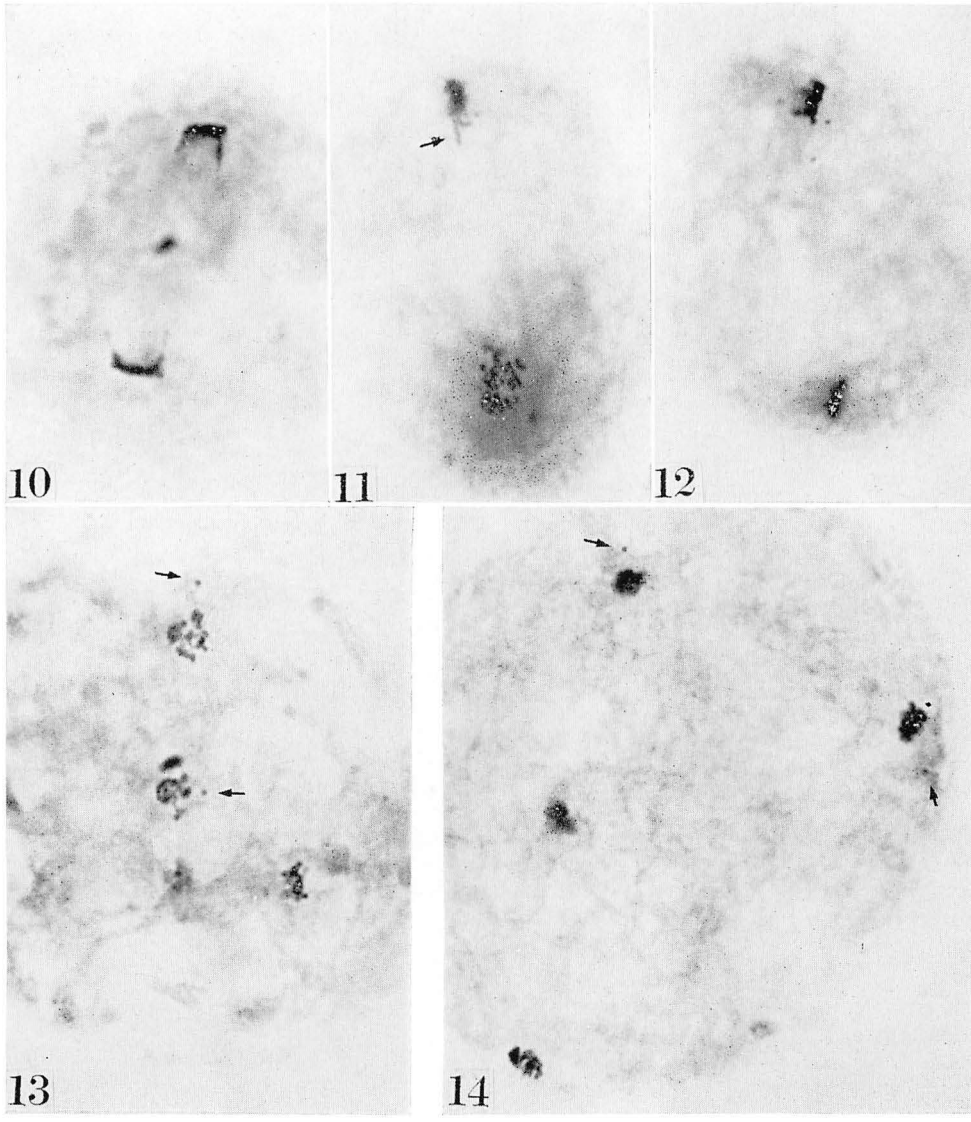
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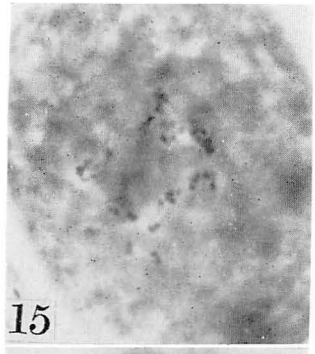
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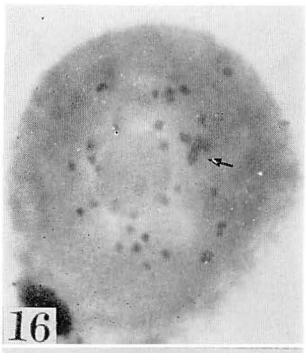
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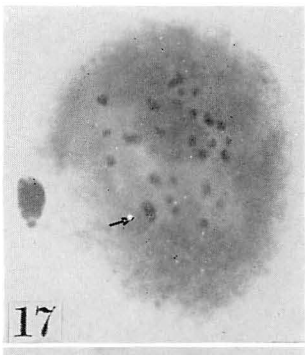
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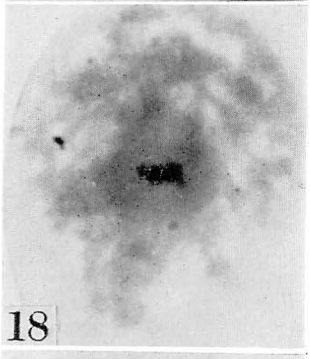
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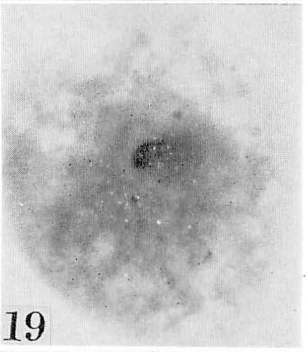
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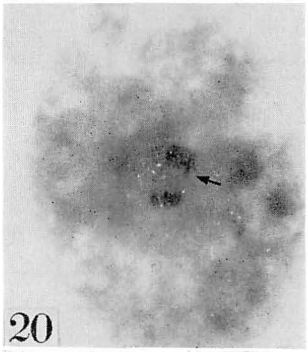
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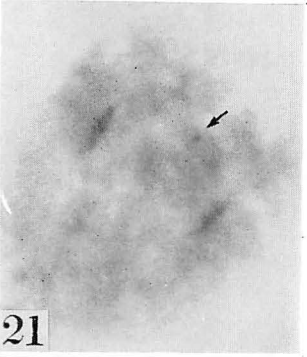
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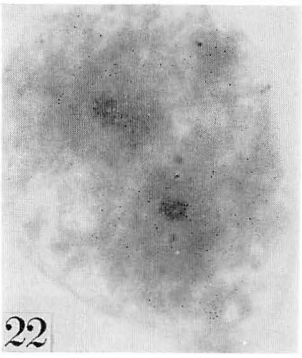
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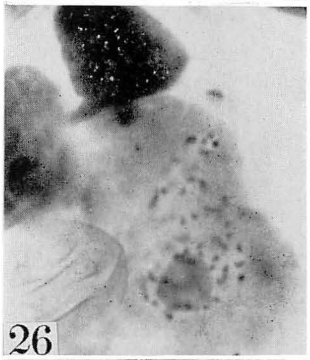
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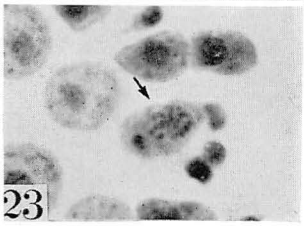
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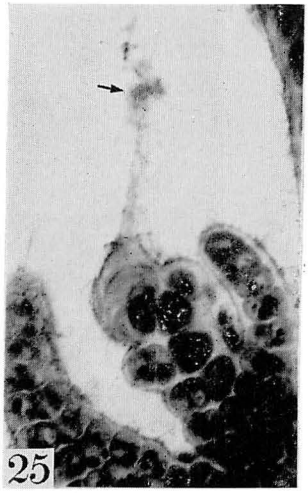
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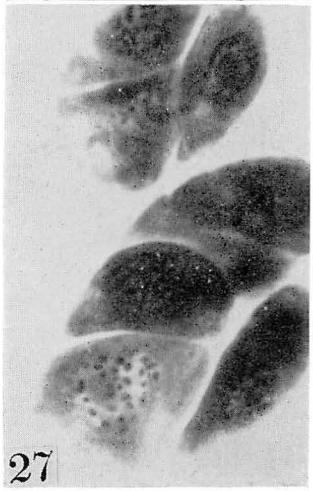
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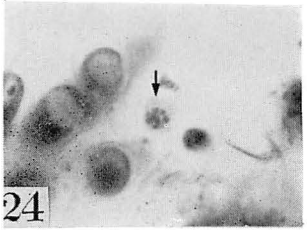
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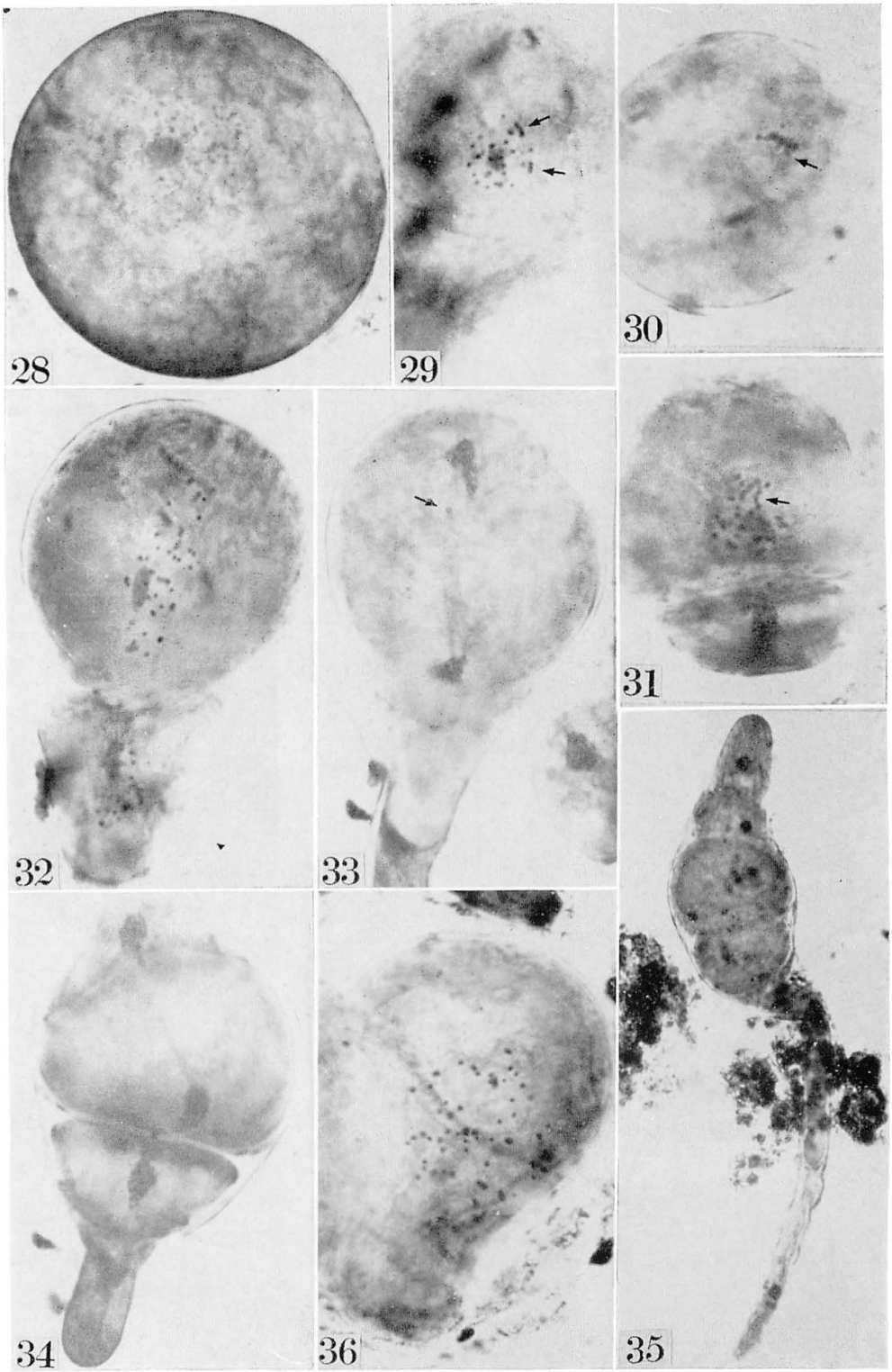
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