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| Title | The Problem of Male Heterogamety in the Decapod Crustacea, with Special Reference to the Sex-Chromosomes in <i>Plagusia dentipes</i> de Haan and <i>Eriocheir japonicus</i> de Haan (With Plate VIII and 7 Textfigures) |
| Author(s) | NIIYAMA, Hidejiro |
| Citation | 北海道帝國大學理學部紀要, 5(4), 283-295 |
| Issue Date | 1937-06 |
| Doc URL | http://hdl.handle.net/2115/27005 |
| Type | bulletin (article) |
| File Information | 5(4)_P283-295.pdf |



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The Problem of Male Heterogamety in the Decapod
Crustacea, with Special Reference to the Sex-
Chromosomes in *Plagusia dentipes* de Haan
and *Eriocheir japonicus* de Haan

By

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(With Plate VIII and 7 Textfigures)

A number of memoirs, dealing with the sex-chromosomes, have already been published on various kinds of Crustacea, as those on Copepoda by Häcker ('08), Braun ('09), Matschek ('09 and '10), Amma ('11), Kornhauser ('15) and Heberer ('32), on Amphipoda by Palmer ('25 and '26), and lastly on Decapoda by Fasten ('14), Delpino ('34) and Leopoldseder ('34). Unfortunately, however, in all these recorded cases demonstrations of the sex-chromosomes seem to the present author to be inconclusive, and therefore the problem of heterogamety still remains unsolved, in connection with the chromosomes in question, so far as the class Crustacea is concerned.

The author, who has been studying the chromosome morphology of Crustacea, following the suggestion of Prof. Kan Oguma, during the past few years, has recently discovered the sex-chromosomes in two species of decapod Crustacea, *Plagusia dentipes* and *Eriocheir japonicus*. They are composed of X and Y in the male cells and are not only distinguishable from each other by their size difference but also are discriminable from autosomes by their behaviour during meiosis.

The investigation has been carried on at the Mitsui Institute of Marine Biology under the direction of Prof. Kan Oguma, to whom the author wishes to express his sincere gratitude for his kind guidance and important suggestions. Hearty thanks are also due to Dr. Sajiro Makino, who gave continuous help and valuable advices during the entire course of the study. The author is greatly indebted

to Dr. Tane Sakai, the Shimoda Marine Biological Station of Tokyo Bunrika Daigaku, from whom the author was able to learn all about the taxonomy of crabs.

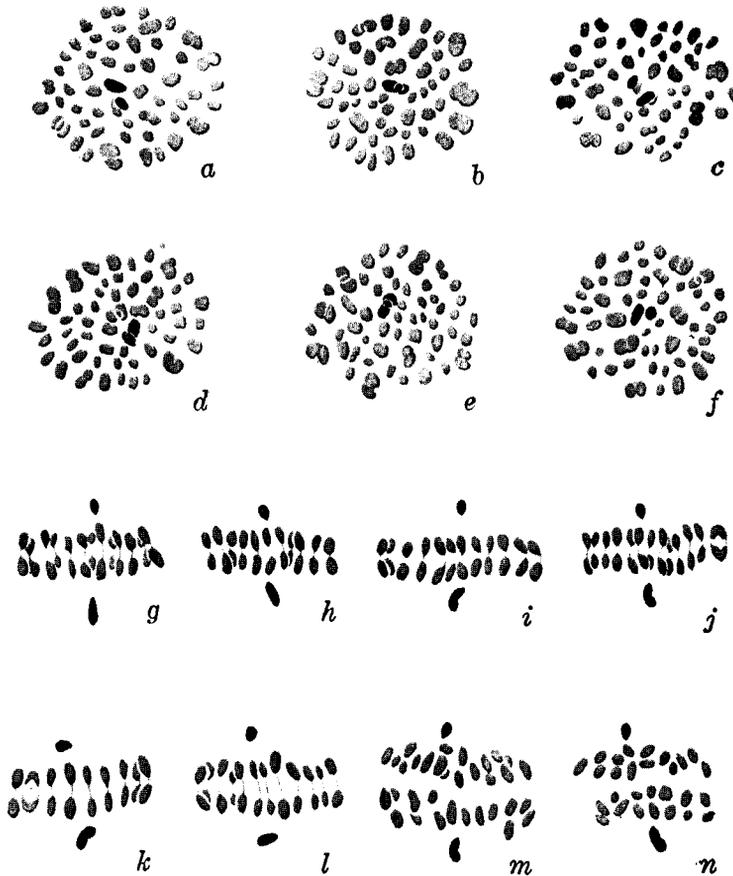
Material and Method

Plagusia dentipes de Haan is a reddish crab with a carapace about 5 cm. in breadth. It is commonly found along rocky shores of the southern parts of the Japanese Pacific coast. *Eriocheir japonicus* de Haan, on the other hand, is sharply characterized by having tufts of long hairs on its chelae. It is distributed all over Japan as an inhabitant of fresh or brackish waters as in the rivers, streams or river mouths. The crabs, from which the material of the present work was obtained, were collected from shores near the Mitsui Institute at various times during October 1935 and March 1936 in the case of the former species and in May 1936 in the case of the latter species. The testes were taken out in living state immediately after capture and put into fixative as soon as possible. Flemming's weak solution showed the best result for preservation of the chromosomes in favorable condition. For staining, iron haematoxylin method after Heidenhain was exclusively employed. Detailed treatment for preparation is quite similar to what described in the author's previous paper (Niiyama, '34).

Observations

I. *Plagusia dentipes* de Haan

The primary spermatocyte. As the division of the primary spermatocytes takes place synchronously, at least in those contained in one cyst, it is not difficult to find the dividing figures of cells, in which the chromosomes are seen in favorable condition to study, in a considerable number. Viewed from a pole of division it seems very clear that a metaphase plate is made up of 52 bivalent chromosomes or tetrads, distributed evenly throughout the whole area of the plate (Textfigs. 1, *a-f*; Figs. 1 and 2 in Pl. VIII). Size differences are noted among them in a rather remarkable degree, and the larger ones at least acquire a median transverse split in accordance with the cases previously observed in different kinds of Decapoda by the present author ('34, '35 and '36). When examined closely, how-



Textfig. 1. Metaphase chromosomes of primary spermatocytes of *Plagusia dentipes*; *a-f*, polar-view, 52 tetrads and XY complex in each. *g-n*, side-view. The XY or sex-chromosomes are indicated in deep black. $\times 3700$.

ever, it will immediately be noticed that every one garniture of chromosomes involves in reality two more in addition to the 52 mentioned above, of which the additional two are not arranged on the same level with the 52 tetrads, thereby being entirely invisible so long as attention directed to that level. They first become evident only when the focus is changed gently upward or downward from where the tetrads lie, since they take their position separately on both sides of the equatorial plate. When one of them is visible

under a certain focus the other one is completely hidden from vision, and *vice versa*, but both of them invariably occupy the central part of the spindle. It is of particular importance that two such chromosomes are found constantly in every cell of the same dividing phase. To examine their morphology in detail attention is particularly called to the side view of the spindle, of which eight examples are reproduced (Textfig. 1, *g-n*; Figs. 3-10 in Pl. VIII).

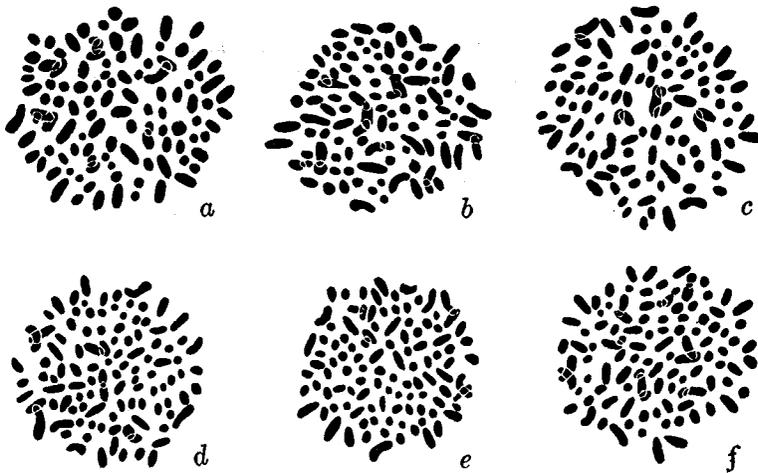
As is readily recognizable from these figures, the two chromosomes in question are not the tetrad, which always appear like dumb-bells with median constrictions, but evidently belong to the rod-chromosomes of univalent nature from the morphological point of view. They differ from each other in their relative magnitude, one being approximately $2/3$ as long as the other one, and this ratio seems to be definite without variation. Sometimes a constriction is observable at the middle portion of the longer one (Textfig. 1, *i, j, k, m* and *n*). Their central position in the spindle is clearly recognized again in such figures of spindles in profile, lying probably on a line connecting the two poles of the spindle at the center of the latter. At the confronting ends they become gradually tapered and more or less pointed at the tips, while the opposite ends are rounded. Such a structure strongly supports the view that these two distinct chromosomes might have been connected together at the pointed ends to form a bivalent at a certain period prior to the stages reproduced in the figures, but become separated precociously as compared with ordinary tetrads. If the present view be established, then the metaphase plate of the primary spermatocyte is essentially made up of 53 bivalents, of which one however gives up the connection of its component halves at a far earlier time than the remaining autosome tetrads which separate at anaphase (Textfig. 1, *m* and *n*).

From the facts stated above, these chromosomes are neither tetrads displaced by some mechanical causes nor chromatoid bodies, but the sex-chromosomes, X and Y, the former being represented by the larger one while the latter by the shorter one, considering their behaviour and morphology, in parallel to cases investigated in other kinds of animals. For example, precession of the X and Y chromosomes in a similar manner has clearly been observed in Neuroptera by Oguma and Asana ('32), Kichijo ('34) and Naville

et de Beaumont ('33). It follows that in the present species the male has heterogametic nature in respect to the sex-chromosomes.

Thus the chromosome constitution of the primary spermatocyte should be $52 + XY = 53$; this number actually does represent the haploid number of the present species.

The spermatogonium. In contrast to three cases previously studied, *Cambarus*, *Paralithodes* and *Panulirus* (Niiyama, '34, '35, '36), the spermatogonia of the present species do not undergo simultaneous division in those contained within a cyst, but dividing cells are encountered intermingled with the resting cells. At metaphase chromosomes constitute an equatorial plate of nearly round circumference, and do not overlap but are separated from one another. The primary and secondary spermatogonia may be classified according to their size (Textfig. 2, *a-c* primary, *d-f* secondary spermatogonia).

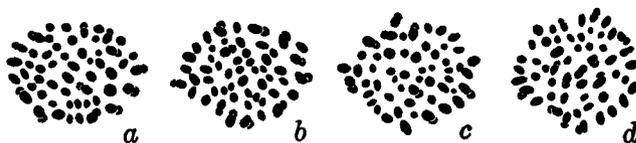


Textfig. 2. Metaphase chromosomes of spermatogonia of *Plagusia dentipes*, 106 chromosomes in each. *a-c*, primary spermatogonia. *d-f*, secondary spermatogonia. $\times 3700$.

After careful counting in many equatorial plates of spermatogonia it is proved without doubt that the diploid number of chromosomes is 106. The chromosomes of the spermatogonial garniture show isomorphic nature; all chromosomes similarly have terminal fibre attachment and vary in length from rods to spherules. It seems impossible to identify the homologous pairs, because of the considerably

large number of chromosomes with slight differences. The same reason, furthermore, prevents the identification of the X and the Y chromosomes already observed in the primary spermatocyte.

The secondary spermatocyte. The equatorial plate of the secondary spermatocyte is remarkably reduced in dimension as well as the chromosomes themselves, compared with those of the primary spermatocyte although they have a strong resemblance in appearance with the latter. In the polar view of the equatorial plate, the chromosomes in dyad structure always count 53 without difficulty (Textfig. 3, *a-d*). Theoretically speaking there should be present two



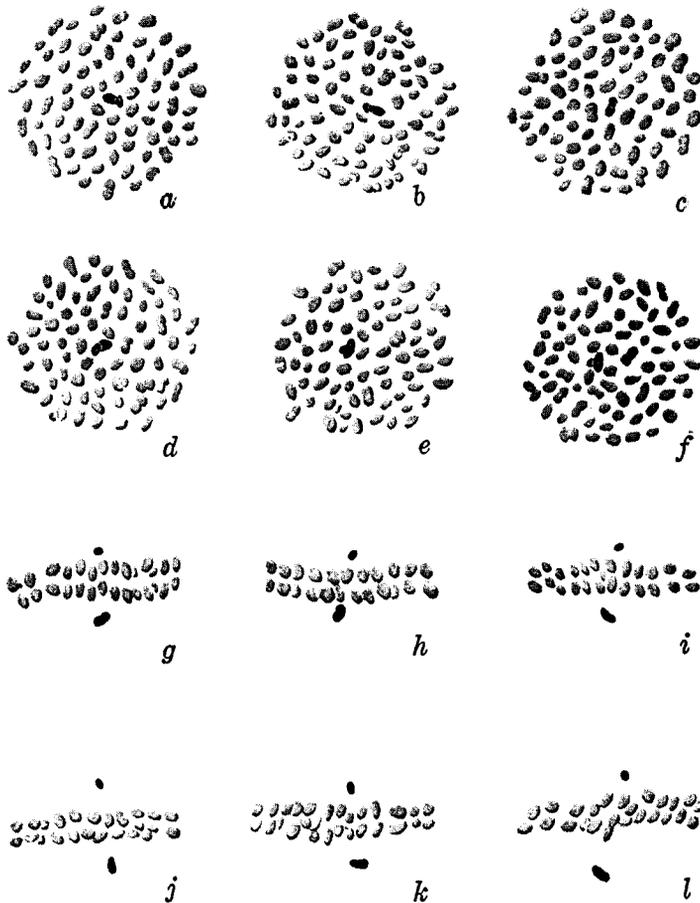
Textfig. 3. Metaphase chromosomes of secondary spermatocytes of *Plagusia dentipes*, 53 dyads in each. $\times 3700$.

kinds of secondary spermatocytes, of which one contains the X chromosome and the other involves the Y. But their distinction is practically impossible for the same reason as stated in the case of the spermatogonial chromosomes.

II. *Eriocheir japonicus* de Haan

The primary spermatocyte. The dividing figures of the primary spermatocytes could be observed abundantly in a certain cyst as in the case of *Plagusia dentipes*. An equatorial plate is composed of 73 tetrads in an arrangement similar to the preceding case, as shown in polar observation of it (Textfig. 4, *a-f*). The size difference is developed in less degree among these tetrads as compared with the former species, but the median transverse sutures are also observable in those of larger size. It is worthy of notice that in this species, the two particular chromosomes are to be discovered again on both sides of the metaphase plate holding a definite distance from the latter, it being possible to catch their images only when the foci are changed from the equator. They take also the central position in the metaphase plate and show a marked difference in their relative

size. The smaller one is about one half the larger one in length. Sometimes, a constriction appears at the middle portion of the larger one of these chromosomes. Compared with the corresponding chromosomes of the preceding species, precession seems to occur in less



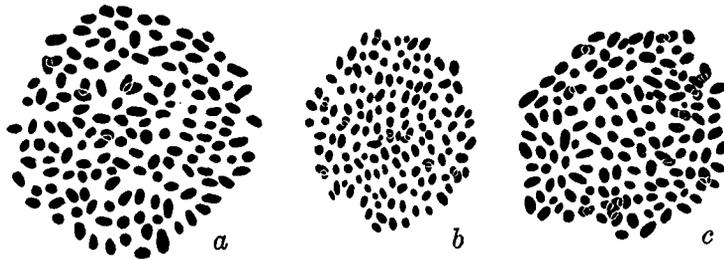
Textfig. 4. Metaphase chromosomes of primary spermatocytes of *Eriocheir japonicus*; a-f, polar view, 73 tetrads and XY complex in each. g-l, side-view. The sex-chromosomes are indicated in deep black. $\times 3700$.

degree in the present species, a fact which the side view of the metaphase plates obviously shows (Textfig. 4, g-l and Figs. 16-20 in Pl. VIII). These particular chromosomes in question should also

be considered the X and the Y of sex-chromosomes according to their size respectively.

The chromosome formula of the primary spermatocyte are therefore $73 + XY = 74$, constituting the haploid number of the present species.

The spermatogonium. Dividing figures of spermatogonia are found along the walls of cysts which involve only spermatozoa in their central portions, in a quite similar manner to that observed in *Plagusia dentipes*. In the polar view of metaphase plate, the chromosomes appear in extreme clearness as shown in Textfigure 5, *a-c*. By their size, the primary and the secondary spermatogonia may also be distinguished (Textfig. 5, *a* and *c*, primary spermatogonia; *b*,



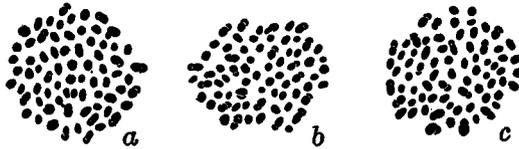
Textfig. 5. Metaphase chromosomes of spermatogonia of *Eriocheir japonicus*, 148 chromosomes in each. *a-c*, primary spermatogonia; *b*, secondary spermatogonium. $\times 3700$.

secondary spermatogonium). In every equatorial plate examined, 148 chromosomes are invariably counted, as showing the diploid number of the present species. The spermatogonial garniture shows isomorphic nature; all the chromosomes assume short rod-shape or spherular form having the terminal attachment of the spindle fibres. They vary in size in a less degree as compared with the case of the former species. Accordingly, an identification of the sex-chromosomes, as well as that of autosomes, will be attempted in vain by means of comparison of their size. In brief, the chromosome constitution of the present species shows more resemblance in appearance to that of the *Cambarus* and *Cancer* (Fasten, '14, '18, '24) than to that of *Plagusia dentipes*.

The secondary spermatocyte. The equatorial plate is constituted of 74 double-structured chromosomes having an appearance similar

to those of the primary spermatocyte, but they are certainly of dyad nature and of much reduced size (Textfig. 6, *a-c*).

There are presumably present two kinds of garnitures in respect to the kind of the sex-chromosomes, as the one involves the X and other the Y, although each has the same number of chromosomes.



Textfig. 6, Metaphase chromosomes of secondary spermatocytes of *Eriocheir japonicus*, 74 dyads in each. $\times 3700$.

But the distinction of these two kinds is absolutely impossible, so long as the identification of the X and the Y chromosomes remains in obscurity.

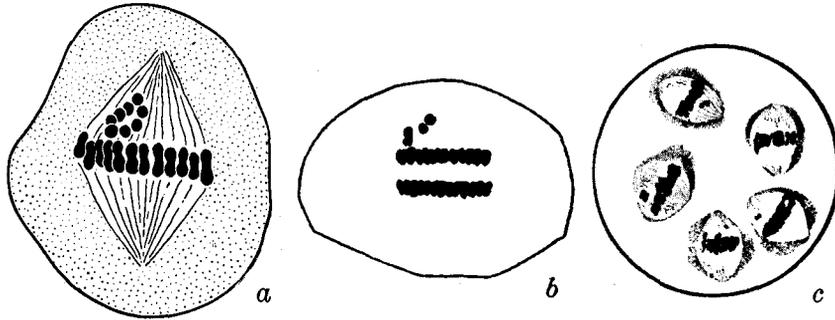
General Considerations

Although the problem of the sex-chromosome of Crustacea has often been discussed during these thirty years, unfortunately no data, definitely establishing heterogamety, have been obtained from any species of this class investigated. There are at least twelve memoirs, in which the occurrence of the sex-chromosome is reported either in the male or in the female germ-cells, as enumerated at the beginning of the present paper. The authors of these memoirs invariably and simply consider that the chromosomes protruding abnormally from the equatorial plate at the first maturation division may be the sex-chromosomes. Neither in behaviour nor in structure, however, are the so-called sex-chromosomes furnished with valid evidences comparable with those found in the other kinds of animals, particularly those of Insecta, the near relatives of Crustacea.

So far as the Decapods are concerned the author is aware of three papers in which the sex-chromosomes are reported. Fasten ('14), in the first place, informed the accessory chromosomes from an American cray-fish, *Cambarus immunis*(?). According to his descriptions, in the metaphase of the first maturing division a group of eight chromosomes is found distinctly separated from the remaining chromosomes, constituting the equatorial plate of the primary

spermatocyte. Such a group of chromosomes orients at the periphery of the spindle and seems to be enclosed within a clear vacuole (Textfig. 7, *a*). In the advanced stage of division the grouped chromosomes remove, without division or separation, towards one pole of spindle, while the remaining chromosomes segregate normally. Fasten ('14), however, did not trace the entire course of the behaviour of the chromosome group in question during meiosis, and avoided any conclusion that these chromosomes are actually the accessories.

Leopoldseder ('34), in the second place, came to the conclusion, after investigation of *Pandalus borealis* and of some species of *Munida* and *Nephrops*, that the heterochromosomes are represented



Textfig. 7. *a*, side-view of primary spermatocytes of *Cambarus immunitis* (?) (Fasten, '14); *b*, side-view of secondary spermatocyte of *Pandalus borealis* (Leopoldseder, '34); *c*, side-view of primary spermatocyte of *Telphusa fluviatilis* (Delpino, '34).

by two distinct ones in his material. They are nearly equal in size assuming a dumb-bell shape with a median constriction, and are arranged separately from the garniture of ordinal chromosomes at the dividing phase of the secondary spermatocyte (Textfig. 7, *b*).

In *Telphusa fluviatilis* Delpino ('34) finally proved the occurrence of an X-group, composed of two sex-chromosomes, which exhibits a peculiar behaviour during the meiosis (Textfig. 7, *c*). That group affords an appearance similar in form as well as in behaviour to Leopoldseder's questionable chromosomes.

The present author rather believes that the sex-chromosomes mentioned in all these three papers based upon three different kinds of Decapoda are those displaced mechanically from their ordinary

position with the remaining chromosomes, due probably to distortion of spindle fibres influenced by fixatives. At least conclusions should be postponed until more detailed history of chromosomes will have been explored than has yet been demonstrated.

In striking contrast to the doubtful cases above mentioned, the finding in two species of crabs, *Plagusia dentipes* and *Eriocheir japonicus*, both of which belong to the family Grapsidae, is extraordinarily clear and is sufficient to establish male heterogamety. The following points will emphasize the view of the present author who believes in the existence of X-Y type of sex-chromosomes in the crabs of the family Grapsidae: (1) the chromosome number of the spermatogonium is without exception even, (2) the X and the Y chromosomes take a definite position in the equatorial plate—they always occupy the central position in the spindle, unlike chromatoid bodies which are found outside of the latter, (3) these two chromosomes show a definite relative magnitude, (4) their construction is not quadripartite as tetrads, but bipartite as univalent dyads, (5) they separate from each other at anaphase of the first maturing division, much in advance of autosome segregation, or show precession according to another explanation, and (6) they are not to be interpreted as accidentally displaced chromosomes but normal and constant occurrences in auxocytes, since in the every cell they afford a definite configuration and behaviour.

The reason why the present author has failed to find the sex-chromosomes in his previous studies on *Cambaroides japonicus*, *Paralithodes camtschatica* and *Panulirus japonicus*, may probably be due to the fact that their differentiation is not yet accomplished either in morphology or in behaviour. In fact, the precession of sex-chromosomes evidently differs in grade in the two studied species, as it is much more advanced in *Plagusia dentipes* than in *Eriocheir japonicus*. If the separation of sex-chromosomes is still less retarded than the latter case, then there arises at last a case in which they are practically impossible of distinguishment from the ordinary chromosomes, and the three species previously investigated by the present author will be examples of such a case.

Summary

1. The X-Y type of sex chromosomes is discovered in two species of Grapsidae, *Plagusia dentipes* and *Eriocheir japonicus*, in their primary spermatocyte. Precession of the sex-chromosomes is observed, at the same time, in both the species. The chromosome constitution of the primary spermatocyte is $52 + XY$ in the former species and $73 + XY$ in the latter.

2. The spermatogonium contains 106 chromosomes in *Plagusia dentipes* and 148 chromosomes in *Eriocheir japonicus*. The X and the Y chromosome could not be identified among the spermatogonial chromosomes in either species.

3. The secondary spermatocyte shows 53 dyads in *Plagusia dentipes* and 74 dyads in *Eriocheir japonicus*. Two kinds of secondary spermatocyte could not be distinguished in either species, in consequence of obscurity of size difference between the sex-chromosome and autosomes.

4. Theoretically considered from the mode of division of sex-chromosomes at the first maturing division it is beyond doubt that heterogamety actually occurs in the male sex.

Literature

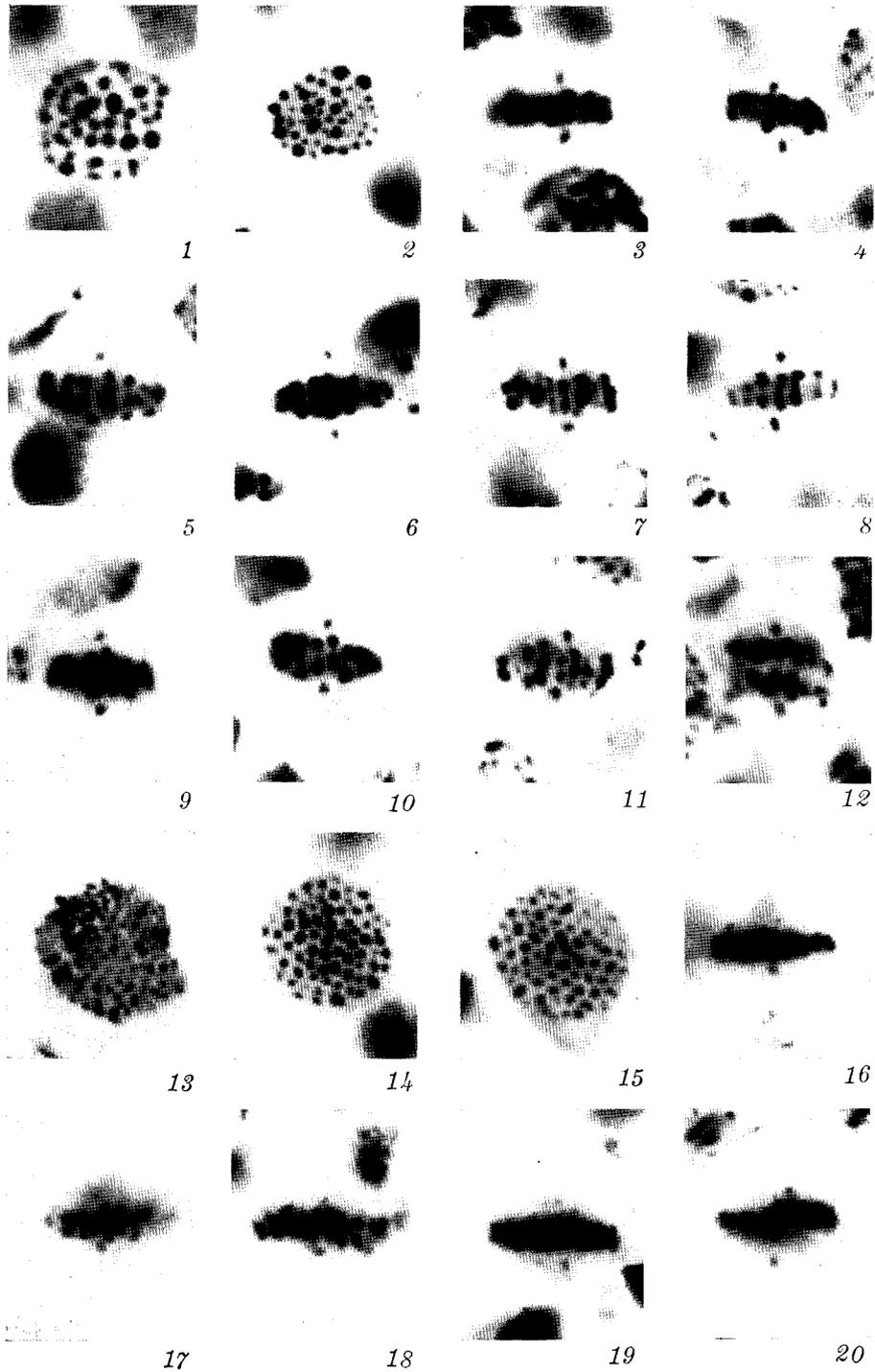
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Explanation of Plate VIII

Photomicrographs taken under magnification 2400 ×.

- Figs. 1-2. Metaphase polar view of primary spermatocyte of *Plagusia dentipes*.
- Figs. 3-10. Metaphase side view of primary spermatocyte of the same species.
- Figs. 11-12. Anaphase side view of primary spermatocyte of the same species.
- Fig. 13. Metaphase polar view of spermatogonium of *Eriocheir japonicus*.
- Figs. 14, 15. Metaphase polar view of primary spermatocyte of the same species.
- Figs. 16-20. Metaphase side view of primary spermatocyte of the same species.
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