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CYTOTAXONOMY AND SEX-DETERMINATION  
IN THE CRUSTACEA

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## I. INTRODUCTION

The present investigation was undertaken with a hope to discuss the bearing of cytology upon the mechanism and processes of evolution, and to make a new approach to classical problems of morphological taxonomy in certain groups of the Crustacea. At present, it is generally accepted that, so far as the higher animals are concerned, all evolutionary transformations have had their origin in the chromosomes, and that they furnish the material source of evolutionary changes, since they constitute the physical basis of heredity (White '57a). Therefore, any significant alteration in the structure and behaviour of the chromosomes represents an evolutionary change. The differences in chromosome numbers, and chromosome patterns which frequently distinguish one species from its relatives throw new light on the problem of taxonomy. These considerations will reasonably also be applicable for lower animals such as Crustacea.

The problem how and why related species of organisms have acquired visibly different chromosome sets, or karyotypes, in the course of their evolution, has long attracted the prime interest of cytologists. The progress of genetics has led to the understanding that each of the characters of an organism represents the results of the interaction of genes (White '54). It should be stated therefore that the nature of organisms will be more exactly mastered from the viewpoint of cytological knowledge involving chromosome constitution than from the morphological standpoint. Cytologists are concerned with the cytotaxonomic differences which exist between related species. Such differences may sometimes be used to distinguish sibling or cryptic species that cannot be separated on the basis of merely external characters. They are the results of chromosomal rearrangements which have arisen spontaneously and established themselves in the course of phylogeny. The cytogenetic data thus far presented have enhanced the development of cytotaxonomy which is based upon cytological and genetical criteria (White '57b).

As early as 1885, the cytological study of Crustacea was initiated by Carnoy. He reported the chromosomes of four species of Crustacea. Probably due to poor fixation, however, he could not determine the precise number of chromosomes in those species. Following Carnoy's study, a considerable number of papers have been published on the chromosomes of various species of Crustacea. The report pertaining to animal chromosomes published by Makino ('56) indicates that the morphology of chromosomes of about 150 species covering seven orders of Crustacea have been reported by a number of investigators.

In spite of the considerable amount of work done on the chromosomes of Crustacea, knowledge on those chromosomes has remained very limited, remarkably less progress having so far been made in this group of animals than in other groups of Arthropoda, *e. g.* Insecta. Reference to the literature shows that the majority of earlier works with respect to the chromosome morphology of Crustacea, have reported very meagre results, due probably

to the technical difficulties. It is evident that basic data for understanding the evolution of the hereditary mechanism in Crustacea are incomplete. Thus, the need for more exact knowledge of the chromosome morphology in various groups of Crustacea is increasing for the sake of the establishment of the scientific taxonomy of this group of animals. In order to make some contributions to this object, the present author has undertaken a comparative study of the chromosomes in three orders of Crustacea: Decapoda, Isopoda and Amphipoda, with particular regard to the karyological relationship to systematics, under the suggestion and guidance of Dr. Sajiro Makino.

The present paper will describe in the following pages the results obtained in this chromosome survey, with discussion on the data presented by earlier investigators and with particular concern to the interrelationships of taxonomy.

The present study is divided into three parts. Part I is devoted to the investigation of the morphology of chromosomes in 33 species covering three orders of Crustacea. Beginning with the work of Carnoy on the chromosomes of Crustacea, which appeared as early as 1885, a considerable number of works have successively been published in various species covering several orders of Crustacea by many investigators. The majority of earlier works are not satisfactory from present-day-standards of cytology. To obtain good preservation of chromosomes of Crustacea is a task very difficult, because of the fact that they are marine dwellers. The preservation of chromosomes of marine animals is a matter of difficulty in general. Further, many crustacean species have a comparatively large number of chromosomes which are generally small in size. To advance knowledge of crustacean chromosome morphology a considerable time has necessarily been devoted in the present study due mainly to technical difficulty, to discover the exact morphology of chromosomes.

In Part II will be described the relationship between chromosome morphology and taxonomy of the Crustacea. Chromosome numbers vary considerably among the different orders. Further, the chromosome numbers vary notably among the subdivisions of the order. Attention in this study was directed to the problem of whether any relation exists between chromosome number and taxonomical classification considering the evolution of chromosome complexes.

Part III deals with the sex-determining mechanism in Crustacea in relation to the chromosomes. Following the pioneer work of Braun ('09) and Matschek ('09, '10) on the sex-chromosomes of Copepoda, many reports have been issued on the sex-mechanism of Crustacea; on Phyllopoda by Baker & Rosof '27, '28; on Ostracoda by Bauer '34, '40 and Dietz '54, '58; on Copepoda by Braun '09, Matschek '09 '10, Heberer '32 and Beervann '54; on Isopoda by Dworak '35 and Steiger & Bocquet '54; on Decapoda by Fasten '14, Leopoldseder '34 and Delpino '34; and on Amphipoda by Palmer '25, '26. The data presented by these authors seem to contain some doubts in more or less degree as considered from present-day-standards. Attention in this study was directed to the question whether sexual difference of chromosomes would really exist and, if they do exist, what type of

sex-mechanism of the Crustacea would occur in relation to the other groups of animals.

Before going further, the author's hearty thanks are due to Emeritus Professor Kan Oguma for his valuable advices. Also, the author wishes to acknowledge here his very great indebtedness to Dr. Sajiro Makino, Professor of Zoology, Faculty of Science, Hokkaido University, who has offered continuous guidance and encouragement, for his kindness in revising and improving this manuscript. Further the author's thanks should be extended to Dr. Saburo Saito, Professor of Zoology, Faculty of Fisheries, Hokkaido University, who has given strong support in various ways to the completion of the present investigation.

## II. MATERIAL

The following 33 species of crustaceans provided the material for the present study. They are classified into three Orders:

### ORDER DECAPODA

Suborder Natantia	
Group Penaeidea	
Family Penaeidae	<i>Penaeus japonicus</i> (BATE)
Suborder Reptantia	
Group Palinura	
Family Palinuridae	<i>Panulirus japonicus</i> (V. SIEBOLD)
Group Astacura	
Family Potamobiidae	<i>Cambarus clarkii</i> (GIRARD)
Family Nephropsidae	<i>Cambaroides japonicus</i> (DE HAAN)
	<i>Nephrops japonicus</i> TAPPARONE-CANEFRI
	<i>Nephropsis carpenteri</i> WOOD-MASON
Group Anomura	
Tribe Galateidea	
Family Galateidae	<i>Cervimunida princeps</i> BENEDICT
Tribe Paguridea	
Family Paguridae	<i>Eupagurus ochotensis</i> (BRANDT)
Family Coenobitidae	<i>Coenobita rugosa</i> H. MILNE-EDWARDS
Family Lithodidae	<i>Paralithodes camtschatica</i> (TILESIUS)
	<i>Paralithodes platypus</i> BRANDT
Group Brachyura	
Tribe Oxystomata	
Family Carapidae	<i>Matuta lunaris</i> (FORSKÅL)
Family Leucosiidae	<i>Philyra pisum</i> DE HAAN
Family Raninidae	<i>Ranina ranina</i> (LINNÉ)
Tribe Brachygnata	
Superfamily Oxyrhyncha	
Family Majidae	
Subfamily Inachinae	<i>Machrocheira kaempferi</i> DE HAAN
Superfamily Brachyrhyncha	
Family Portunidae	
Subfamily Portuninae	<i>Ovalipes punctatus</i> (DE HAAN)
Subfamily Lupinae	<i>Scylla serrata</i> (FORSKÅL)
Family Atelecyclidae	<i>Telmessus cheiragonus</i> (TILESIUS)
Family Xanthidae	<i>Atergatis floridus</i> LINNÉ
Family Grapsidae	
Subfamily Grapsinae	<i>Pachygrapsus crassipes</i> RANDALL
Subfamily Varuninae	<i>Hemigrapsus sanguineus</i> (DE HAAN)
	<i>Hemigrapsus penicillatus</i> (DE HAAN)
	<i>Eriocheir japonicus</i> DE HAAN
	<i>Gaeticus depressus</i> (DE HAAN)
	<i>Sesarma intermedia</i> (DE HAAN)
	<i>Plagusia dentipes</i> DE HAAN
Subfamily Sesarminae	
Subfamily Plagusiinae	

## ORDER ISOPODA

Suborder Oniscoidea	
Family Ligiidae	<i>Megaligia exotica</i> (ROUX)
Family Tyliidae	<i>Tylos granulata</i> MIERS
Suborder Valvifera	
Family Idoteidae	<i>Cleantiella isops</i> (GRUBE)
	<i>Idotea japonica</i> RICHARDSON
Suborder Flabellifera	
Family Sphaeromidae	
Subfamily Sphaerominae	<i>Cymodoce japonica</i> RICHARDSON
	<i>Tecticeps japonicus</i> IWASA
<b>ORDER AMPHIPODA</b>	
Suborder Gammaridea	
Family Gammaridae	<i>Anisogammarus annandalei</i> (TATTERSALL)

## III. TECHNIQUE

For the study of chromosomes, the testes which were removed from animals in living state immediately after capture were exclusively adopted as material. After several trials in fixation, the present author was successful in finding a new fixative which is a modified formula of weak Flemming's mixture for the purpose of such a study as the present investigation. This fixative proved the most excellent so far tried for preservation of the chromosomes of every species of Crustacea. The contents of this mixture are as follows: -

2% Osmic acid	5 c.c.
1% Chromic acid	25 c.c.
0.1% Acetic acid	10 c.c.
Distilled water	60 c.c.

The testes were allowed to rest in the above fixing fluid for 20 to 24 hours; the material was washed thoroughly in running water for the same length of time. After being dehydrated with ascending grades of alcohol in the usual way, the material was cleared in creosote-toluol and toluol, then embedded in paraffin. Sections were cut 10 micra in thickness. After bleaching with hydrogen peroxide, the sections were stained by the iron-haematoxylin method after Heidenhain.

All the figures were drawn with Abb's drawing apparatus, using a Zeiss H. I. 90 obj. and a K20× compensating ocular, at the level of the desk on which the microscope was set. The magnification rate of all the figures was 3700 times in diameter.

IV. PART I. COMPARATIVE MORPHOLOGY OF CHROMOSOMES  
IN THIRTY-THREE SPECIES OF CRUSTACEA

## ORDER DECAPODA

1) *Penaeus japonicus* (BATE)

The present species is an edible prawn famous in Japan for its delicious taste. It

belongs to to the Family Penaeidae, Penaeidea, Natantia of the Order Decapoda. The prawns from which the material of the present study was obtained, were captured in shallow water at Kisarazu, Chiba Prefecture by fishermen in May, 1943.

Though the dividing figures of spermatogonia were scanty among the testicular cells, a few good metaphase equatorial plates could be observed. Fig. 1 is an illustration of them. In the polar view, the chromosomes appear taking a usual radial arrangement forming a circular equatorial plate. After careful analysis of chromosomes, it was found that the diploid complex consisted of 14 V-shaped metacentric elements and 78 acrocentric ones showing a gradual diminution of size from rod to dot. The metacentric chromosomes are arranged into 7 homologous pairs, according to their sizes and shapes. They all have nearly submedian attachment of the spindle fibres. It is very difficult to determine the homologous pairs among the acrocentric chromosomes, except for several larger ones. The diploid number of this species, therefore, was ascertained to be 92 which are formulated as  $14V' + 78R'$  in the male.

Division takes place synchronously in all spermatocytes within a cyst. The dividing figures of spermatocytes, therefore, could be observed in large number. Figs. 2 and 3 are the polar views of primary spermatocyte at metaphase. The number of bivalents in an equatorial plate is 46 without exception. Several greater bivalents assume criss-cross or triangular shape considered as a modified form of the former tetrad. Remaining smaller bivalents take the form of a dumbbell or rectangle. At the side view of anaphase, all the bivalents separate synchronously into two equal halves. There is no chromosome having a character in form and behaviour usual to the sex-determining chromosome.

Fig. 4 is a polar view of a secondary spermatocyte at metaphase. There are 46 chromosomes of dyad nature. Seven of them are V-shaped elements and the remaining ones are rod- and dot-shape. These 7 V-shaped dyads are evidently the descendants of V-shaped chromosomes in the spermatogonial garniture. At anaphase they divide synchronously into equal monads. Also at this division no particular chromosome in either form and behaviour could be observed.

The chromosome number of the present species is 92 ( $14V' + 78R'$ ) in diploid and 46 in haploid.

## 2) *Panulirus japonicus* (V. SIEBOLD)

This species is well known in our country as a tasty edible spiny lobster of the Family Palinuridae, Palinura, Reptantia of the Order Decapoda. The lobsters from which the material of the present work was obtained were collected by nets in waters adjacent to the Mitsui Institute of Marine Biology at Suzaki, near Shimoda, Izu Peninsula, at intervals from March to August in the years, 1933 and 1935.

In the testis, the cells contained in a cyst are of the same phase; some cysts are filled with only spermatogonia, while others are provided with spermatocytes of different kinds, primary or secondary.

The spermatogonial chromosomes assume a rosette form in their metaphase arrange-

ment, as they lie radially in the equatorial plate. They are found well apart from one another with sharply defined contour, so as to make the counting of chromosome number very easy in spite of their large number. In order to determine the number and form of the chromosomes, many equatorial plates were examined from their polar aspect. Examples are given in Figs. 5 to 6, every one of which overlapping of chromosome occurs to a slight degree. After careful counting, it was decided that the diploid complex consists of 140 chromosomes. As is recognizable in the figures, the spermatogonial garniture is of polymorphic nature; of 140 chromosomes, 12 are V-shaped metacentric elements while the remaining ones are all acrocentric rods showing gradual diminution of size. According to the size and shape the metacentric chromosomes are arranged into 6 homologous pairs, of which three pairs are rather large as compared with the remaining three. They all have nearly submedian attachment of the spindle fibres. Among the acrocentric chromosomes, it seems impossible to know which two's constitute the homologous pairs, except for some larger ones.

The chromosomes of the primary spermatocytes are observed with absolute clearness in the polar view of the metaphase plate, as they scatter evenly and are distinctly separated from each other. As the usual case may be, the larger ones take the peripheral position with those of smaller size lie in the central region of the spindle. As expected from the count of the spermatogonial chromosomes, a primary spermatocyte contains invariably 70 bivalent chromosomes (Figs. 7-8). Of the large sized bivalents found in the peripheral part of the spindle, three are sharply distinguishable from the others in having a striking characteristic in their morphology. They belong to the compound ring-tetrads, composed of two or three rings connected in a series with metacentric fibre attachment. It is certain that these bivalents are the descendants of three pairs of larger V-shaped chromosomes in the spermatogonium. Three more bivalents with metacentric fibre attachment, but small in size, are always found in addition to those just mentioned. They are evidently descendants of three pairs of smaller metacentric chromosomes in the spermatogonium, but whether they have ring structure is not known at present. When viewed from the pole of division, the remaining bivalents exhibit, at least in larger ones, a dumbbell shape being different in size, with a remarkable transverse suture in each middle region. In the smaller bivalents, however, the actual presence of the middle suture is not proven for certain. In the anaphase of the first division, the separation of chromosomes always takes place synchronously.

As naturally expected from the mode of separation of chromosomes in the first division, only one kind of secondary spermatocyte is produced in respect to the chromosome complex. There are also counted 70 dyads without exception in the metaphase equatorial plate of the secondary spermatocytes (Fig. 9). The chromosomes are quite similar in arrangement to those of the spermatogonia and primary spermatocytes, as larger ones are arranged at the periphery of the spindle while dot-like ones are in the central region. Further, all chromosomes corresponding to those of the spermatogonium are to be pointed out too in the

garniture of the secondary spermatocytes : 6 V-shaped metacentric chromosomes and the remaining acrocentric ones from rod to dot shape.

The chromosome number of the present species is 140 in diploid and 70 in haploid in the male. The polymorphic nature of the chromosome complex consisting of the formula  $12V' + 128R'$ , is a remarkable character of the species. Such a condition of chromosomes is very interesting from the morphological viewpoint, when one considers the evolution of the chromosome complex in Crustacea. There is no chromosome to be considered as the sex-chromosome in form and behaviour.

### 3) *Cambarus clarkii* (GIRARD)

The present species is a crayfish originally introduced from America in 1930 and propagate abundantly throughout Japan in paddy-fields. It belongs to the Family Potamobiidae, Astacura, Reptantia of the Order Decapoda. The materials for the present study were obtained from some specimen gathered from a paddy-field near Ohfuna Railway Station, Kanagawa Prefecture in June, 1936.

In the testis many dividing figures of spermatogonia could be observed. Fig. 10 is an example of metaphase polar views of spermatogonia. As seen in the figure, the chromosomes distribute homogeneously well apart from one another with sharply defined contour in the equatorial plate. The number and form of chromosomes could easily be observed in spite of their large number. After careful counting, it was decided that the diploid complex consists of 192 chromosomes. As in the case of the previous species, *Panulirus japonicus*, the present species also is polymorphic in diploid garniture; of 192 chromosomes, six are V-shaped metacentric elements, while the remaining ones are all acrocentric rods showing gradual diminution of size. Size differences between metacentric and larger acrocentric chromosomes are not so remarkable as in the case of the previous species, *Panulirus japonicus*. According to their sizes and shapes the metacentric chromosomes are arranged into 3 homologous pairs. They all have nearly submedian attachment of the spindle fibres. Since the acrocentric chromosomes show gradual diminution of size, it seems impossible to know which two's constitute the homologous pairs, except for some of the larger ones.

Dividing figures of spermatocytes could be observed abundantly within a cyst. At the polar view of metaphase of a primary spermatocyte chromosomes are observed with absolute clearness, as they distribute well apart from one another forming a round equatorial plate. Fig. 11 is an example of polar view of a primary spermatocyte at metaphase. The number of bivalent chromosomes contained in an equatorial plate is invariably 96. The haploid number of the present species is 96 in the male. When viewed from the pole of division, almost all the bivalents take dumbbell shape form showing a transverse suture in each middle region. Among these bivalents there are a few assuming the shape of a criss-cross or analogous form. Certainly these bivalents are the descendants of three pairs of V-shaped chromosomes in the spermatogonium. Remaining small bivalents are in the form of a rectangle. At anaphase of first division, all the bivalents divide synchronously

into two equal halves and migrate to each pole of the spindle. There is no chromosome particular in form and in behaviour to be considered as the sex-chromosome, nor does any chromosomes group make strange movement as happened to occur in *Cambarus immunis* (?) according to Fasten ('14).

As expected from the mode of the first division, there is only one kind of secondary spermatocyte. Polar view of the metaphase equatorial plate of the secondary spermatocyte is shown in Fig. 12. In the circular equatorial plate there occur 96 chromosomes in dyad nature. Among these, there are three metacentric chromosomes, having submedian fibre attachment and the remaining 93 are acrocentric rod- and dot-shaped chromosomes. At the second division, all the chromosomes divide simultaneously into two equal halves. In this division also no particular chromosomes in form or in behaviour could be detected.

The chromosome number of the present species is 192 in diploid having the formula  $6V' + 186R'$ , and 96 in haploid in the male. There is neither any chromosome which is in behaviour characteristic to the sex-chromosome, nor any chromosome group taking a strange course at division.

#### 4) *Cambaroides japonicus* (DE HAAN)

The present species is a common crayfish in lakes and streams throughout Hokkaido. It belongs to the Family Potamobiidae, Astacura, Reptantia of the Order Decapoda along with the previous species, *Cambarus clarkii*. The material with which the present study was carried out was mostly obtained in the suburbs of Sapporo, Hokkaido but some were secured from Lakes Tōya and Kussharo, Hokkaido, during June, July and August 1933. The material obtained from the beginning to the end of July proved to be most favourable for the study of chromosomes of various stages.

The spermatogonium is rather large of size and nearly round in shape. Two kinds of spermatogonia, the early and late can be distinguished by their size. The spermatogonium does not undergo simultaneous division in a cyst; the dividing figures are found independently. In the polar view, the chromosomes are noticed to be distributed homogeneously throughout the whole area of the equatorial plate, arranging themselves well apart from one another. It is not very difficult, therefore, to count the number of chromosomes in spite of their large number. Careful counting of many equatorial plates revealed that the spermatogonium contains invariably 196 chromosomes (Figs. 13-14). The chromosomes are mostly short straight rod-shaped, but sometimes slightly curved. They are however, all acrocentric chromosomes. The present species, therefore, has a chromosome garniture of isomorphic nature. It seems to be impossible to identify the homologous pairs except for the longest one. These longest chromosomes always form a pair, being nearly equal in size; they can be easily distinguished from the others.

Fasten ('14) reported 200 dot-like chromosomes to occur in the spermatogonium of *Cambarus virilis*, stating that "the chromosomes are spherical in shape". The present species, therefore, is remarkably different from *Cambarus virilis*, in respect to the shape of

chromosomes, though the two forms do have an isomorphic complex of chromosomes.

In the primary spermatocyte the chromosomes arrange themselves with extreme clearness in the metaphase plate as shown in Figs. 15 to 16. Therefore, counting of the chromosome number is rather easy in the present stage. In every equatorial plate examined, a garniture of the haploid chromosomes is, without exception, made up of 98 bivalents constituting a nearly round equatorial plate. The bivalents are generally so disposed in the equatorial plate as to be scattered about throughout the whole space of the latter. Frequently, a small vacant space is found in the central region of the equatorial plate as seen in Figs. 15 and 16, probably due to some mechanical effects in preparation of the sections. When viewed from a pole, almost all the bivalents exhibit a conspicuous transverse suture across their middle region, as a result of which they assume a dumbbell shape. At anaphase, all the bivalents separate symmetrically into two equal halves. The division of the chromosome took place synchronously, neither precession nor succession could be observed in any chromosome. Any particular group of chromosome taking a strange behaviour which was reported by Fasten ('14) to occur in *Cambarus immunis* (?) could not be observed in the present species.

There is only one sort of secondary spermatocyte concerned with the chromosome complex. The chromosomes and the equatorial plates are generally much smaller in size, compared with those of the primary spermatocyte. Careful counting shows, without any doubt, 98 chromosomes of dyad nature in every metaphase plate (Fig. 17). They are all rod-shaped but seem very much shorter than the spermatogonial chromosomes and exhibit dyad nature superimposing the constituent halves with each other. There is nothing particular in the mode of chromosome separation in the present division, every dyad separating equally into two daughter monads. Fig. 18 shows one of the two daughter chromosome garnitures at anaphase in which 98 monads are clearly to be counted. In this group of chromosomes one finds a long chromosome, which is clearly distinguishable from the remaining ones. This chromosome, as may be readily noticed, is that one which corresponds to one of the longest pair found in the spermatogonium. The reason why this large chromosome has never been observed in either primary or secondary spermatocyte divisions, is that the difference in size between the latter mentioned chromosome and the remaining ones is not sufficiently great to distinguish it in a state otherwise than its full extension. The longest chromosome is not considered to be the sex-element, since it divides equally in the maturation divisions and there is nothing unusual in the behaviour.

The chromosome number of the present species in the male is 196 in diploid. They are all of acrocentric rod-shape. The haploid number is 98. Throughout the dividing stages of meiosis, no chromosomes peculiar in heterotropic behaviour characteristic to the sex-chromosome could be observed.

##### 5) *Nephrops japonicus* TAPPARONE-CANEFRI

The present species is a deep-sea edible prawn of the Family Nephropsidae, Astacura, Reptantia of the Order Decapoda. The prawns for the present work were obtained in

April, 1937 from Suruga Bay, to the west of the Izu Peninsula, at depths of 200–400 meters. It is distributed in the deep-sea along the coast of the Pacific of southern Japan from the Bōsō Peninsula to Shikoku.

In the testis, dividing figures of spermatogonia are rather rare. Several excellent figures could be observed among the cells constituting the inner walls of cysts. Fig. 19 is an example of the metaphase polar view of the spermatogonium. As seen in the figure, chromosomes constitute a round equatorial plate distributing themselves well apart from one another with sharply defined contour, so as to make counting of the chromosome number very easy in spite of their large number. The spermatogonial graniture of the present species is isomorphic in nature: all the chromosomes are acrocentric showing gradual diminution from rod to dot in shape. After careful counting, it was decided that the diploid complex consists of 164 chromosomes. There are no chromosomes having particular form. The diploid number of this species, therefore, was determined as 164 in the male.

The division of the primary spermatocyte takes place synchronously within a cyst. The author failed to observe any dividing figures of secondary spermatocytes in the present material. Fig. 20 is a polar view of a primary spermatocyte at metaphase. The bivalents are observed with absolute clearness in the plate, as they scatter evenly and are distinctly separated from each other. About a half of all the bivalents assume the usual type of compact dumbbell shape observed in Decapoda in general. The remaining ones possess a strange quadripartite structure, each consisting of well differentiated quadripartite chromatic elements. The existence of these bivalents will be dealt with in detail in the following species, *Nephropsis carpenteri*. The number of bivalents in the present species in an equatorial plate is 82 without exception. There is present no chromosome taking particular behaviour considered as the sex-chromosome in the primary spermatocyte (Fig. 21). Nevertheless the dividing figure of the secondary spermatocyte could not be observed in the present material. It is supposed from the above results that only one kind of secondary spermatocyte having 82 dyads is produced.

The chromosome number of the present species is 164 in diploid and 82 in haploid in the male. The chromosomes are all acrocentric in nature showing an isomorphic complex. There is not any evidence for the presence of any particular chromosome to be considered as the sex-chromosome, either in respect to behaviour or in structure.

#### 6) *Nephropsis carpenteri* WOOD-MASON

The present species is also an edible prawn inhabiting the deep-sea. The present form, together with *Nephrops japonicus*, belongs to the Family Nephropsidae. The two prawns are therefore closely related taxonomically. The prawns obtained at the same place as the former materials furnished the material for the present study.

Dividing figures of spermatogonia are very rare in the testes of the present material. Two excellent metaphase polar views were fortunately obtained for close study. An example is given in Fig. 22 which shows a minor degree of overlapping of chromosomes. Counting indicates that the diploid complex consists of 152 chromosomes. The

spermatogonial garniture is polymorphic in nature : of 152 chromosomes, 30 are metacentric V-shaped elements, while the remaining 122 are all acrocentric rods showing gradual diminution of size from rod to small dot. It is impossible to identify with confidence the homologous pairs, except for some comparatively large V-shaped chromosomes and some small dot-shaped ones. As a whole, the metacentric V-shaped chromosomes occupy a peripheral position in the equatorial plate surrounding the rod-shaped acrocentric ones in the central part.

The chromosomes of primary spermatocytes are observable clearly in the polar view of the metaphase plate. Since the bivalents are found well apart from one another, counting of their number and observation of their morphology was rather easily carried out. Every equatorial plate shows 76 bivalents with unvarying clearness (Figs. 23-24). Almost all the bivalents with exception of a few smaller ones, exhibit strange quadripartite structure. These bivalents are the same in structure as those observed in the previous species, *Nephrops japonicus*. Observations from a pole show that the quadripartite bivalents are composed of four chromatins attaching two by two. The constituent element of each chromatid mass show uniform size. Each masses of a bivalent are connected with two fine threads. Considered from the constitution of the tetrad, this quadripartite structure is the that of the upper half of a tetrads. There must be, therefore, another like quadripartite structure under it attaches closely to it. In other words, a bivalent is composed of eight smaller chromatid elements, of which each four are connected with chromatic fibres. Such a structure is seen in several bivalents among usual quadripartite bivalents. These strange bivalents appear to be hexapartite in structure. It seems to be an opened shape of the above bivalent at one end. When observed at anaphase, the octopartite structure appears in side view of the first division (Fig. 25). Such a structure of bivalents may be due to a loose condensation of chromonema at metaphase of the primary spermatocyte. At anaphase of the first division, no chromosome having special behaviour and to be regarded as the sex-chromosome could be observed.

The chromosome number of the present species in the male is 152 having a formula of  $30V' + 122R'$  in diploid and 76 in haploid. On the basis of the fact that almost all the bivalents of the present species and the closely related species, *Nephrops japonicus*, possess a similar quadripartite structure in a half of all the bivalents, it seems probable that the two species under consideration have a close systematic relationship. Further, the chromosome constitution of the closely related two species, *Nephrops japonicus* and *Nephropsis carpenteri*, 164 (all rods) for the former,  $152 (30V' + 122R')$  for the latter, is specially interesting when one considers the evolution of the chromosome complex.

#### 7) *Cervimunida princeps* BENEDICT

The present species is a deep-sea crab, belonging to the Family Galateidae, Galateidea, Anomura, Reptantia of the Order Decapoda. The specimens for the present work were obtained from the same place and at the same time as those of the previous two species, *Nephrops japonicus* and *Nephropsis carpenteri* in April, 1937 from Suruga Bay to the west

of the Izu Peninsula, at depths of 200 to 400 meters.

Dividing figures of spermatogonia were observable among cells constituting the inner walls of testicular cysts. Fig. 26 is an example of well-preserved metaphase plates of spermatogonia. Counting revealed that the diploid complex consists of 18 V-shaped metacentric elements and 91 rod- and dot-shaped acrocentric ones, showing the diploid number of 109 in the male. The odd number, 109 clearly suggests the existence of a sex-chromosome. The acrocentric chromosomes are odd in number and therefore contain any unpaired elements, but they vary in size gradually from rod to dot; the condition makes it impossible to identify any unpaired elements.

The division takes place synchronously in all spermatocytes within a cyst. The dividing figures of spermatocytes, therefore, could be observed in a large number. Every primary spermatocyte under study showed, in side-view, three univalent chromosomes, two being at one side and the other one at the other side of the equatorial plate; the three lie a considerable distance apart from the remaining chromosomes which arrange themselves in an equatorial plate. The number of bivalents lying on the equatorial plate was found to be 53. Size differences are noted among them in a rather remarkable degree; some larger ones exhibit the type of ring- and cross-tetrads, while the remaining ones are of dumbbell shape. The larger bivalents may be the descendants of V-shaped chromosomes in the spermatogonium. By changing the focus of the microscope from the equatorial plate up and down, two chromosomes at one side and one chromosome at the other side, or *vice versa*, could be observed in every metaphase plate as shown in Figs. 27 and 28. According to the general rule of the sex-determining mechanism, it is reasonable to assume that these three univalent chromosomes constitute a compound sex-chromosome complex composed of  $X_1X_2$ - and Y-elements. The  $X_1$  and  $X_2$  are nearly identical in size, while the Y is slightly larger than either of the two X's (Figs. 31 to 33). The Y is joined separately at one end with each of the two X's. At metaphase, the compound sex-chromosome is always situated considerably off the equatorial plate without exception. It seems therefore that the distance pairing of the sex-trivalent is established by a pairing mechanism during the diakinetik stage, but is not due to the mechanism of precession at metaphase. A comparable case was reported in the male of a neuropteran, *Plethosmylus decoratus* by Hirai ('56). The chromosome formula of the primary spermatocyte of the present species is represented by  $53+X_1+X_2+Y$ . At anaphase, the two X's and Y separate from each other and migrate to opposite poles together with daughter halves of ordinary chromosomes. The number of chromosomes in the daughter complexes after division is 54 and 55, respectively (Fig. 34, a-b).

The secondary spermatocytes subsequently produced are of two sorts: the one possesses 54 chromosomes and the other 55 (Figs. 35-36). In the secondary spermatocyte, it is difficult to distinguish the sex-chromosomes due to the absence of any character distinguishable from those of the autosomes. Further, there are no precocious chromosome in the second division.

In the following, the behaviour of the sex-trivalent will be traced in detail during the meiotic prophase stages. In the leptotene nucleus of the meiotic prophase the sex-chromosomes made their appearance distinguishable from the autosomes. In this stage, two compact chromatic masses which are positively heteropycnotic could be observed among lightly stained spireme threads in the nucleus. One of them is composed of two parts of nearly equal size : they connect with each other transversely. The other one is about  $1/2$  the size of the above double mass having no connection therewith (Figs. 29-30). In view of the heteropycnotic character in the leptotene stage and considered from their shape and size observed at metaphase, it seems most probable that the double mass consists of the  $X_1$  and  $X_2$  in association, whilst the smaller one is the Y. Since there is no nucleolus in any stage of the meiotic prophase, the association of the sex-chromosomes with the nucleolus was not observable. With the advance in the stage of the meiotic prophase, there appear many chromatic bodies in the nucleus indistinguishable in size from the  $X_1$ ,  $X_2$  and Y; this makes impossible the distinction of the sex-chromosomes from the autosomal chromatic bodies. Though there is no evidence, the establishment of the distance pairing of the sex-elements might have taken place in late diakinetik stage, as has been reported to occur in *Plethosmylus decoratus* (Hirai '56).

In conclusion the diploid number was found in the present species to be 109 forming a complex of  $18V' + 91R'$ . In the primary spermatocyte, 53 bivalents and the  $X_1X_2Y$ -trivalent were observed. There are two kinds of secondary spermatocytes having 54 and 55 chromosomes, respectively. During the meiotic prophase two X's and Y make their appearance as distinct chromatic bodies in the auxocyte. The  $X_1X_2Y$ -trivalent displays a distance pairing in the spindle of the primary spermatocyte metaphase. The sex-mechanism of  $X_1X_2Y$ -type as found in the present species is quite unique in the Crustacea, so far as the chromosome survey has gone.

#### 8) *Eupagurus ochotensis* BRANDT

This species is a common hermit-crab showing a wide distribution in the shallow sea all around Japan. It belongs to the Family Paguridae, Paguridea, Anomura of the Order Decapoda. The materials of the present study were obtained in shallow waters adjoining the Akkeshi Marine Biological Station, Hokkaido by dredging in June, 1934.

Many dividing figures of spermatogonia were observed in the testes. The early spermatogonia were used for observation to avoid mistake in counting the number of chromosomes, since they are so large in number. Fig. 37 is an example of metaphase equatorial plates of early spermatogonia. The chromosomes constitute a large round equatorial plate distributing themselves well apart from one another with sharply defined contour. Overlapping of chromosomes occurs to a slight degree. In spite of their large number, therefore, counting of chromosomes is not very difficult. After careful counting, it was decided that the diploid complex consists of 254 chromosomes. The spermatogonial garniture is polymorphic in nature : of the 254 chromosomes, 18 are metacentric V-shaped ones while the remaining ones are all acrocentric rod- and short dot-shaped. Those 18

metacentric V-shaped chromosomes are sorted into 9 pairs by comparing their sizes and shapes. Each of them has a nearly submedian attachment of the spindle fibres. In the acrocentric rod-shaped chromosomes, it was impossible to sort the homologous pairs, except for several larger ones. As a whole, the larger chromosomes occupy a peripheral position in the equatorial plate surrounding the smaller one in the central portion.

Division of the spermatocytes takes place approximately simultaneously within a cyst. Many dividing figures, therefore, could be observed in the testis. The chromosomes of a primary spermatocyte constitute a round equatorial plate; they scatter well apart from each other (Figs. 38-39). Careful observations of many equatorial plates at metaphase made it clear that every metaphase plate contains 127 bivalent chromosomes without exception. All the bivalents take a dumbbell shape excepting some larger ones which appear as criss-cross tetrads. They may be the descendants of 9 pairs of metacentric V-shaped chromosomes in the spermatogonia. At anaphase, each of the bivalents divides synchronously into two equal halves. Dividing figures of the secondary spermatocytes are fewer than those of primary spermatocytes. Every secondary spermatocyte shows at metaphase 127 chromosomes of dyad nature constituting a circular equatorial plate (Fig. 40). There is only one kind of secondary spermatocytes. Several larger ones occur as the descendants of V-shaped chromosomes in the spermatogonia. In division, all the elements divide synchronously without showing any element of unusual behaviour.

In conclusion, the present species is characterized by the diploid number of 254 constituting a formula of  $18V' + 236R'$  and showing the haploid number of 127. This chromosome number is the greatest so far reported among animals.

#### 9) *Coenobita rugosa* H. MILNE-EDWARDS

The present species is a terrestrial hermit-crab, living in southern Islands of the Japan. It belongs to the Family Coenobitidae, Paguridea, Anomura of the Order Decapoda. Specimens captured on Chichijima of the Bonin Islands in July, 1937, provided the material for study.

Though testes of several individuals were sectioned and above 200 preparations are investigated, there could be found no dividing figure except a solitary excellent metaphase plate of the early spermatogonia in the testes as given in Fig. 41. The equatorial plate is very large in dimensions; the chromosomes distribute evenly well apart from each other. This makes easy the counting of the chromosomes. After careful counting the number of chromosomes is decided as 230. They are all of acrocentric rod-shape showing gradual diminution of size from rod- to minute dot-shape. The present species has an isomorphic complex of chromosomes. It is rather difficult to sort the homologous pairs in the diploid complex. The present observations could not include any of haploid chromosomes, since there were no dividing figures in the testes. The chromosome number of the present species, 230, ranks second in the animal chromosomes so far as the scope of the present chromosome survey is concerned.

#### 10) *Paralithodes camtschatica* TILESIIUS

The present species is a well-known edible crab widely distributed along the coasts of the north Pacific Ocean. It belongs to the Family Lithodidae, Paguridea, Anomura of the Order Decapoda. The material consists of testes from several specimens captured by nets from the waters adjoining the Akkeshi Marine Biological Station, Hokkaido, in the middle part of May, 1934. They were operated and fixed immediately after capture on board the fishing boats.

The spermatogonia are found constituting the inner walls of the seminal tubules, the central portions of the latter being occupied by cells of various kinds in maturing stage. The spermatogonia do not undergo simultaneous division in a cyst, but dividing figures are encountered independently among the resting cells. As seen in the polar view of the metaphase, the chromosomes arrange themselves well apart from one another, forming a fairly round equatorial plate. It is not very difficult, therefore, to count the number of chromosomes, in spite of their considerably large number. By careful counting of many clear plates, the diploid number was determined to be 208 (Figs. 42-43). The chromosomes range in length from rod to dot, showing gradual diminution in size. The larger rod-shaped chromosomes are sometimes curved. These chromosomes seem to be metacentric V-shaped ones but by comparison of several metaphase complexes, it was decided that all the chromosomes are acrocentric ones.

In the metaphase polar view of primary spermatocytes, the bivalents appear with distinct clearness, scattered evenly on the equatorial plate, as drawn in Figs. 44-45. In the present stage, therefore, the number of chromosomes can be counted with much ease and without mistake. By counting many clear metaphase plate, it was ascertained that every primary spermatocyte contains 104 bivalents without exception. The bivalents vary in size, and when viewed from the pole, the majority of the larger ones exhibit a clear dumbbell shape, each one showing a conspicuous transverse suture in the middle region. Because of this suture each bivalent seems to be composed of two equal halves. In the smaller bivalents, however, one can hardly demonstrate the actual presence of that middle suture. In the ensuing division, the bivalents are all separated equally into two sister components. The separation of the sister chromosomes is quite synchronous in every bivalent.

The symmetrical distribution of chromosomes in the primary spermatocyte division gives rise to only one kind of secondary spermatocyte in respect to the chromosome garniture. The metaphase equatorial plate of the secondary spermatocyte much resembles that of the primary spermatocyte, but is reduced in dimensions as compared with the latter. The individual chromosomes exhibit a distinct dual nature, they are scattered all over the equatorial plate without any overlapping with one another. In the equatorial plates examined, it is proved that the number of dyads is 104 without any exception (Figs. 46-47). Nothing particular is found in the mode of the secondary spermatocyte division; every dyad divides into two monads.

The chromosome number of the present species is 208 in diploid and 104 in haploid,

probably the same as in *Cambarus immunis* (?) in which Fasten ('14) reported 104 chromosomes as the haploid number. So far as the present observations show, in *Paralithodes camtschatica*, there are to be found no kinds of chromosomes, so particular in form and in behaviour as to be considered as sex-chromosomes.

#### 11) *Paralithodes platypus* BRANDT

This species is an edible crab, having a close relationship to the previous species, *Paralithodes camtschatica*. It is distributed along the coasts of the north Pacific Ocean together with the previous species. It belongs also to the Family Lithodidae, Paguridea, Anomura of the Order Decapoda. The crabs on which the present work was based were obtained by nets in the Okhotsk Sea adjacent to the Kurile Islands. The preservation of the testes was made through the courtesy of Mr. Isamu Takeuchi of the Hokkaido Regional Fisheries Research Laboratory, to whom the author wishes to express his cordial thanks.

The dividing figures are encountered independently among the resting spermatogonial cells. At metaphase, the chromosomes constitute a large circular equatorial plate well apart from one another. It is not so difficult, therefore, to count the number of chromosome notwithstanding their large number. Based on the counting of several clear plates, the diploid number was determined to be 206 (Fig. 48). This number is two less than that of the previous species, *Paralithodes camtschatica*. Remarkable difference between the chromosome garnitures of these two related species, is the existence of metacentric V-shaped chromosomes in the former species. In detail, the chromosome complex of *P. camtschatica* are isomorphic in nature, while *P. platypus* has a polymorphic complex: of 206 chromosomes, 18 are metacentric V-shaped, while the remaining 188 are acrocentric rod-shaped showing gradual diminution of size from long rods to minute dot. The 18 metacentric chromosomes are sorted into 9 homologous pairs according to their sizes and forms, though it is rather difficult to identify the homologous pairs in the 188 acrocentric chromosomes.

The primary spermatocytes divide synchronously in each cyst. In the metaphase polar view of this stage, the bivalents take their appearance with excellent clearness distributing themselves evenly on the equatorial plate (Figs. 49-50). There are 103 bivalents in every equatorial plate without exception. Of these 103 bivalents, several comparatively larger ones take dumbbell form whilst the remaining ones appear as a rectangular in form. The transverse suture across the central portion of each bivalent could not be ascertained in the present species though it was detected rather distinctly in the chromosomes of *Cambaroides japonicus* and *Paralithodes camtschatica*. It may depend on strong condensation of chromonema at this stage. At anaphase, all the bivalents separate equally into two daughter halves and go synchronously to each pole.

Careful counting of many equatorial plates of secondary spermatocytes shows without any exception 103 chromosomes of dyad nature (Fig. 51). Several of them are comparatively larger in size. They may be the descendants of 9 pairs of V-shaped chromosomes as found in the spermatogonia. At anaphase, all the dyads separate equally into two equal halves

simultaneously. No chromosome with any particular characters could be detected throughout either of the reduction divisions.

It is evident from the above observations that the present species possesses 206 diploid chromosomes consisting of  $18V'+188R'$  and 103 bivalents in haploid. The number, 206, is two less than those of the closely related species, *Paralithodes camtschatica*. The present species is characterized by a polymorphic chromosome garniture, but the latter by an isomorphic complex.

### 12) *Matuta lunaris* FORSKÅL

The present specizes is a common sea-shore crab distributed widely along the southern coasts of Japan from Sagami Bay to Formosa. It belongs to the Family Carapidae, Oxystomata, Brachyura, Reptantia of the Order Decapoda. Specimens capture on the sea-shore near the Mitsui Institute of Marine Biology at Suzaki near Shimoda, Izu Peninsula in May, 1935 provided the material for the present study.

Dividing figures of spermatogonia are observed here and there among the resting spermatogonial cells, which constitute the inner walls of seminal tubules. The spermatogonial cells are very small when compared it with those of above described species belonging to the other groups of Decapoda: Palinura, Astacura and Anomura. The cells of the present species are  $1/2$  the diameter of those of *Paralithodes*. Accordingly the number of chromosomes contained in the cells of the present species is very small. After careful observations of several excellent metaphase plates, it was decided that the diploid complex consists of 94 chromosomes. When observed from the pole at metaphase, the chromosomes assume a circular rosette form in arrangement, as the larger chromosomes lie at the periphery with the smaller ones at the central portion of the equatorial plate. Examples are given in Figs. 52 and 53. As is recognizable in the figures, the spermatogonial garniture is of polymorphic nature. Four chromosomes of a metacentric V-shaped and 90 ones having an acrocentric nature, diminishing gradually in size from long rod to minute dot, constitute every spermatogonial garniture. Though the four metacentric chromosomes could easily be paired by their sizes and forms, it is difficult in the remaining 90 acrocentric ones to find the actual partners for pairing, since their size-difference is gradual.

The chromosomes of the primary spermatocytes are observed with absolute clearness in the polar view of the metaphase plate in spite of their small size, as they scatter evenly and are distinctly separated from each other. The number of bivalents contained in every equatorial plate is 47 invariably (Figs. 54-55). The larger ones are dumbbell in shape as commonly occurs in crustaceans. The smaller ones exhibit rectangular form. The transverse suture across the central portion of each bivalent could not be observed in the present species. At anaphase, all the bivalents separate into equal halves simultaneously.

Dividing figures of secondary spermatocytes were observable abundantly. As expected from the mode of separation of bivalents in the previous division, only one kind of secondary spermatocyte is produced in respect to the chromosome complex. Fig. 56 is an example of the metaphase polar view of the secondary spermatocyte which shows 47 chromosomes in

dyad nature.

Based on the above results, it is concluded that the chromosome number of the present species is 94 consisting of  $4V' + 90R'$  in diploid and 47 in haploid. No chromosome, peculiar in shape or in behaviour, was found in either meiotic division.

### 13) *Philyra pisum* DE HAAN

The present species is a small crab, with a carapace about 20 m.m. in breadth. It is common in muddy shallow seas throughout Japan from Iwate Prefecture to Kyûshû. The present study was based on the testicular material derived from several specimens captured in Kisarazu, Chiba Prefecture in May, 1943. It belongs to the Family Leucosiidae, Oxytomata, Brachyura, Reptantia of the Order Decapoda.

Probably due to the unfavorable season the spermatogonial cells are very scarce in every testis. They were found in thin inner walls of the cyst, surrounding the cells in various stages of meiotic divisions and mature spermatozoa at the central portion of the cyst. Accordingly, the dividing figures of spermatogonia are scarce. Fig. 57 is an excellent example of the metaphase equatorial plates of spermatogonia. The dimensions of cells are also as small as those of the preceding species, *Matuta lunaris*. The chromosomes forming every equatorial plate are 114 in number. Four large metacentric chromosomes of V-shape appear in the periphery of the equatorial plate. They can easily be classified into two homologous pairs, but the remaining 110 chromosomes are all acrocentric chromosomes assuming a rod-shape, diminishing gradually in size from rod to dot shape.

The dividing figures of spermatocytes were abundant in the testes. Figs. 58 and 59 are the metaphase polar views of the primary spermatocytes. The bivalents constitute a circular equatorial plate, distributing themselves equally well apart from each other. In every equatorial plate, 57 bivalents are counted without exception. All the bivalent take the form of a rectangle. Neither the transverse suture across the central portion of each bivalent nor the dumbbell shape, common for decapod bivalents, could be observed. In the ensuing division, all the bivalents separate equally into two equal halves.

There is only one kind of secondary spermatocytes as expected from the mode of the first division. In the equatorial plate of the secondary spermatocytes, 57 chromosomes of dyad character distribute evenly arranging themselves in the equatorial plate well apart from each other (Fig. 60). Among them, two chromosomes appear as a double V. They seem to correspond to two pairs of metacentric V-shaped chromosomes in the spermatogonia. The dyads separate equally into two monads at anaphase of the second division.

So far as the present observations show, in *Philyra pisum* there are discovered no special chromosome, so particular in form and in behaviour as to be considered the sex-chromosome. The present species is characterized by the diploid number of 114 having a complex of  $4V' + 110R'$  and the haploid number of 57.

### 14) *Ranina ranina* (LINNÉ)

This is a species of large edible crabs with a carapace about 100 m.m. in breadth, being very famous for its characteristic form and beautiful colouration on the carapace. It

distributes widely from Sagami Bay to the coast of the south Pacific Ocean. The specimens which the present material was based were captured by traps in the waters adjoining the Mitsui Institute of Marine Biology at Suzaki near Shimoda, Izu Peninsula in September, 1936. It belongs to the Family Raninidae, Oxystomata, Brachyura, Reptantia of the Order Decapoda.

The spermatogonia do not undergo a simultaneous division in a cyst: the dividing figures are found independently together with the resting cells. Due to favourable season of collection many dividing figures could be observed in the material. The cell diameter of the present species is comparatively larger than that of the above two species of Brachyura, *Matuta lunaris* and *Philyra pisum*.

The spermatogonial chromosomes assume a rosette form in their metaphase arrangement, as they lie radially against the centre of the equatorial plate, being well-apart from one another with sharply defined contour. Example are given in Fig. 61. Spermatogonial complex is polymorphic in nature containing 106 chromosomes in each. Of them, 24 are metacentric elements of V-shape and the remaining 82 are acrocentric rod-shaped ones. The latter chromosomes vary in shape from long rod to minute dot. According to the sizes and shapes, all the chromosomes are clearly sorted into 53 homologous pairs.

The metaphase equatorial plate of the primary spermatocytes is very large having nearly the same diameter as that of the spermatogonium. The bivalent chromosomes, 53 in number, are observed with extreme clearness in the equatorial plate (Fig. 62). They are arranged in a radial manner distributing well apart at nearly equal distance from one another. As usual the larger bivalents take a peripheral position with those of smaller size in the central region of the spindle. Some of the larger bivalents take a strange form in their morphology. Under close observation it becomes evident that they are a modification in shape of the simple criss-cross tetrad. The other bivalents excepting the above larger ones, have the shape of a dumbbell or rectangle. The bivalents of the former shape show no remarkable suture in their middle region. At anaphase, all the bivalents separate into two equal halves simultaneously.

Actually there is only one kind of secondary spermatocytes in respect to the chromosome complex. Fig. 63 is an example of the polar view of the secondary spermatocyte metaphase. There are 53 dyads without exception in every plate studied. The chromosomes are quite similar in arrangement to those of the spermatogonium and primary spermatocyte, as the larger dyads are arranged in periphery of the spindle while the dot-like ones are in the central region. Further, all chromosomes corresponding to those of the spermatogonium are detected again in the garniture of the secondary spermatocyte: there are 12 metacentric V-shaped chromosomes and the remaining acrocentric ones varying in shape from rod to dot. At anaphase of the second division these dyads divide synchronously into two equal halves.

No chromosomes peculiar in form and shape could be detected throughout the two reduction divisions. The chromosome number of the present species is 106 in diploid and

53 in haploid in the male. The formula is represented by  $24V'+82R'$ .

**15) *Macrocheira kaempferi* DE HAAN**

The present species is well-known for being very large in size among crabs. They are deep-sea dwellers in Japan. The species belongs to the Family Majidae, Oxyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. The crabs, from which the material for the present work was obtained, were collected in April, 1937 from Suruga Bay, to the west of Izu Peninsula, at depths of 200 to 400 meters.

Dividing figures of spermatogonia were abundant in the testes among the resting spermatogonia. Two representations of the metaphase plates are shown in Figs. 64 and 65. Every metaphase equatorial plate shows 106 chromosomes which arrange themselves well apart from one another. Of them, 16 are metacentric V-shaped elements and the remaining ones are of acrocentric type diminishing gradually in size and shape from rod to dot. Those 16 metacentric chromosomes and several of the larger acrocentric ones can be arranged in homologous pairs by their shapes and sizes. As to the shorter rod- and dot-shaped ones, however, it seems impossible to know the actual partners for homologous pairing.

The dimensions of the equatorial plate of primary spermatocytes are smaller than those of the previous species, *Ranina ranina*, but they are greater than those of *Matuta lunaris* and *Philyra pisum*. In diameter it measures about  $1/2$  that of the former species and  $3/2$  times that of the latter two species. The bivalents arrange themselves equidistantly on the equatorial plate, showing no overlapping of chromosomes (Fig. 66). In all plates examined, 53 bivalents are observed without exception. Several of the larger bivalents assume the form of a criss-cross. They may be the descendants of metacentric V-shaped chromosomes in the spermatogonia. The other smaller bivalents are dumbbell or rectangular in shape. The transverse suture across the middle region of each chromosome is obscure in the bivalents of the present species. In the ensuing division, all the bivalents separate into equal halves synchronously. At the circumference of the equatorial plate, there are always about ten or more black bodies nearly similar in size. They were found surrounding the equatorial plate having no relation to the spindle fibre (Fig. 67). These bodies were also found outside the meiotic prophase nuclei lying close to the nuclear membrane. It seems most probably from the above facts that these bodies are not chromosomes but may be chromatoid bodies. As seen in Fig. 67, all the bivalents separate into two equal halves synchronously at anaphase.

At the second metaphase the chromosomes form a circular equatorial plate distributing themselves evenly in it. They are 53 in number and dyad in nature (Fig. 68). There are 8 x-shaped dyads. They may be the descendants of the 8 pairs of V-shaped chromosomes of the spermatogonia. The remaining 45 elements are all acrocentric rod-shaped. The complex of the secondary spermatocyte is just half the spermatogonial complex. At anaphase, the 53 dyads each separate into two equal halves.

During both meiotic divisions, there are to be seen no chromosomes having any peculiar characters in form and in shape. In conclusion the present species is characterized by the chromosome number of 106 in diploid and 53 in haploid in the male. The present species is the same in chromosome number as *Ranina ranina*, but it shows a different complex. The complex consists of 16V' + 90R'

**16) *Ovalipes punctatus* (DE HAAN)**

The present crab is yellow in colouration with dark spots scattered all over the carapace which is about 56 m.m. in breadth. It is distributed widely in Japan from Hokkaido to Ryûkyû, belonging to the Family Portunidae, Brachyryncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. The specimens which furnished material for study were obtained by means of dradging at Kakisaki, Shimoda Bay, Izu Peninsula, in May, 1936.

Dividing figures of the spermatogonia are rather few in number. Figs. 69 and 70 show the metaphase equatorial plates of the spermatogonial divisions. Counting of the chromosome number is rather easy. It showed that the diploid complement consists of 103 chromosomes, of which 4, or 2 pairs, are metacentric V-shaped while the remaining ones are all acrocentric of short rod-shape. The occurrence of the odd number of spermatogonial chromosomes suggests the presence of an unpaired element in this species. But it was quite impossible to detect such an unpaired element in the diploid complex, because of the fact that the 99 acrocentric rod-shaped chromosomes gradually diminish in size from short-rod to minute dot, there being no remarkable characteristics in any chromosomes beyond the length.

The meiotic divisions are found occurring simultaneously in several cysts in the testes. Over one hundred metaphase figures of the primary spermatocyte were observable in a single section. The dimensions of the equatorial plate of primary spermatocyte at metaphase are slightly smaller than those of the preceding species, *Macrocheira kaempferi*. A metaphase polar view shows 51 distinct bivalents which constitute a beautiful circular equatorial plate. The majority of them assume a rectangular or dumbbell shape which is common in the chromosomes of many crustacean species. By changing the focus of the microscope from equatorial plate a little upward or downward, the existence is disclosed of a chromosome which lies in a nearly central portion of the equatorial plate situated always in one side of the latter (Figs. 71-72). Viewed from the side of the equatorial plate, the presence of this peculiar element becomes very evident, being slightly apart from the equatorial plate in which other elements lie, as clearly shown in Figs. 73 and 74. After close examinations, it becomes certain that this particular chromosome is completely present within the spindle of the metaphase. In shape this chromosome is apparently more slender than the ordinary bivalents; it tapers at one end directed toward the equatoiral plate. Considered from its structure, this chromosome is certainly of a dyad nature and not a bivalent. At anaphase, this chromosome does not separate at all and migrates towards one of the poles of the spindle. As a result of this division, one of the daughter complexes

produced contains this element in its complement. From the fact shown in the morphology and behaviour as above observed, it is quite natural to consider that this chromosome having a dyad nature is the X-chromosome, just comparable to that one generally found in Orthopteran insects. Further, the occurrence of the odd number of spermatogonial chromosome confirms the presence of a single X-chromosome. It is remarkable that the X-chromosome always lies in a central position of the spindle. Thus the chromosome constitution of the primary spermatocyte of this species is shown by the formula;  $n=51+X$ .

The secondary spermatocyte metaphase shows chromosomes with dyad nature scattered evenly in a circular equatorial plate. Careful counting of the number indicates that there are two kinds of cells in the secondary spermatocyte with regard to the number of chromosomes. One of them shows 51 chromosomes (Figs. 75-76), while the other 52 (Figs. 77-78). Considered from the distribution of the X-chromosome as it occurred in the primary spermatocyte division, it is apparent that the cell having 52 elements involves in it an X-chromosome. The X element is entirely indistinguishable in the complex for the reason noted in the case of the spermatogonial cell. At anaphase, all the elements segregate synchronously, and as the result there are produced two kinds of spermatids, one with the X and the other without it. Hence the chromosome complexes of the secondary spermatocyte are as follows; (a) no-X-class having 51 chromosomes, and (b) X-class having 52 chromosomes.

In summary, the chromosome constitution of the present species is shown by the formula;  $2n, 103=4V'+98R'+X$ . and  $n=51+X$ . The X-O type of sex-mechanism of the present species is the first to be found in the decapod Crustacea, although the X-Y and the  $X_1X_2$ -Y sex-mechanisms were found to occur in several other decapods.

#### 17) *Scylla serrata* (FORSKÅL)

This species is a large edible crab, 197 m.m. in breadth of carapace and a member of the Subfamily Lupinae, Portunidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. It is distributed widely from Sagami Bay to the coasts of the Indo-Pacific region. The material upon which the present study was based was obtained from shallow waters near Nabeta, Shimoda Bay, Izu Peninsula, in September, 1936.

The present species, together with the preceding *Ovalipes punctatus*, are included taxonomically in the same Family Portunidae, though they belong to different subfamilies: the present species belongs to Lupinae, while the latter to Portuninae. The cells of the present species are smaller in size than those of the latter species. They are nearly the same size as those of *Matuta lunaris* of the Family Carapidae, Oxystomata.

The spermatogonia do not undergo simultaneous division in a cyst, but dividing figures are encountered independently among the resting spermatogonial cells. Two kinds of spermatogonia, the early and late may be classified by their size; the former has twice the size of the latter. Fig. 79 is an example of early spermatogonia and Fig. 80 is that of the late ones. The size-relation of the chromosomes is parallel to the cell-size. The spermatogonial chromosomes are of isomorphic nature, since all the chromosomes are acrocentric rod-

shaped, diminishing gradually in size from long rod to minute dot. The counting of the chromosome number shows that the diploid complement consists of 106 chromosomes. The chromosome number of the present species is just identical with that of the next preceding two species, *Ranina ranina* and *Macrocheira kaempferi*, though they differ constitution from each other in their chromosome complexes.

Division of the primary spermatocyte occurs simultaneously within a cyst. Many dividing figures, therefore, could be observed in the same sections. Bivalents constitute a beautiful circular equatorial plate at metaphase (Figs. 81-82). In a complex, 53 bivalents, all having rectangular shape, arranged themselves evenly on the equatorial plate. They each separate synchronously into two equal halves at anaphase. Since the present species belongs to the same Family Portunidae with the previous species, *Ovalipes punctatus* having the X-O sex-mechanisms, the primary spermatocytes of the present species were fully investigated. No particular chromosome to be considered as the sex-chromosome, however, was found in respect to behaviour or in morphology.

The dividing figures of the secondary spermatocyte were abundant among the primary spermatocytes. In dimensions the equatorial plate of the secondary spermatocyte is very much smaller than the primary spermatocytes. As expected from the mode of the first division, there is only one kind of secondary spermatocytes. As seen in Fig. 83, 53 chromosomes of dyad nature could easily be counted in every equatorial plate. They are all of rod- or dot-shape. At anaphase, they separate into two equal halves. Also no chromosome peculiar in form or behaviour could be observed in the second division.

In fine, it is concluded that the chromosome number of the present species is 106 in diploid and 53 in haploid.

### **18) *Telmessus cheiragonus* (TILESIUS)**

This is a brown crab having short bristles all over the carapace which is about 80 m.m. in breadth. It is distributed widely from Hokkaido to California along the coasts of the North Pacific. It belongs to the Family Atelecyclidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. The specimens which provided the material for the present study were captured in shallow waters at Muroran, Hokkaido in April, 1940.

Dividing figures of spermatogonia were encountered abundantly in a testis scattering here and there among the cells of resting spermatogonia. Figs. 84 and 85 show examples of the metaphase polar views. The chromosomes constitute a circular equatorial plate arranging themselves well apart from one another. By careful counting of many clear plates, the diploid number was determined to be 124. In the peripheral portion of the equatorial plate, 8 metacentric V-shaped ones are observable, though occasionally some of the smaller V-shaped ones are found lying at the central region. According to their sizes and shapes these 8 metacentric chromosomes are sorted into 4 homologous pairs. The remaining 116 chromosomes are all acrocentric in nature, diminishing in size from rod to dots. It seems impossible to arrange these acrocentric chromosomes into homologous pairs, since

there is no distinguishing character beyond length.

The first division occurs synchronously in a cyst. Many dividing figures could be observed in the same cyst. The metaphase equatorial plate is about  $3/2$  times larger than those of the preceding species, *Scylla serrata* in diameter. Every equatorial plate contains 62 bivalents which are well-apart from one another. Larger bivalents take the form of a dumbbell and smaller ones appear rectangular in form. Among these dumbbell shaped bivalents there are a few larger ones which are considered to be the descendants of metacentric V-shaped chromosomes of spermatogonia. They are not very remarkable in appearance, since the size-difference between the smaller V-shaped chromosomes and longest acrocentric rod-shaped ones is not very evident (Figs. 86-87). At anaphase, all the bivalents separate simultaneously into two equal daughter halves.

In cysts next to the primary spermatocyte at division, the second division took place abundantly. In spite of their small size, 62 chromosomes of dyad nature could be easily observed without any doubt at metaphase (Fig. 88). Four dyads are observed having metacentric structure, while remaining 58 are acrocentric rod-shaped chromosomes. At anaphase, separation of all dyads occurs synchronously. Throughout the two meiotic divisions, no chromosome with any peculiar behaviour could be detected.

Based on the above results, it is evident that the chromosome number of the present species is 124 in diploid and 62 in haploid. The diploid complex is formulated as  $8V' + 116R'$ .

#### 19) *Atergatis floridus* LINNÉ

This is a common crab, about 70 m.m. in carapace breadth, inhabiting the rocky sea-shores. It is of the Family Xanthidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. It shows a wide distribution from the Bôso Peninsula, Japan, to the coast of the Indo-Pacific region. The crabs, from which the material of the present study was obtained, were collected from shores near the Mitsui Institute of Marine Biology at Suzaki near Shimoda, Izu Peninsula in November, 1936.

Due probably to the period of collection of the material, almost all the cysts of testes are filled with mature spermatozoa, while spermatocytes in process of reduction division are observed in the small portion of a testis. The dividing figures of spermatogonia are found very rarely. Fig. 89 is the most ideal metaphase equatorial plate so far observed. The chromosomes distribute themselves well apart from one another, overlapping of chromosomes occurs to a slight degree, though they do not show circular metaphase arrangement. There are 104 easily countable chromosomes in this plate. The spermatogonial complex is of polymorphic nature; of the 104 chromosomes, 4 are metacentric V-shaped elements, while the remaining ones are all acrocentric rods showing gradual diminution in size. Those 4 metacentric chromosomes and several of the longer rod ones can be arranged in homologous pairs, by their shapes and sizes. However, it was impossible to find the actual partners among the remaining shorter chromosomes, for there are many

chromosomes having a similar length.

Since the division occurs synchronously in a cyst, primary spermatocytes at metaphase can be observed abundantly. Bivalents are observed with absolute clearness in polar view of every metaphase plate, as they scatter evenly. The dimensions of the metaphase plate are nearly identical with those of *Telmessus cheiragonus*. Examples are given in Figs. 90 and 91, in each of which there are contained 52 bivalents. Two of them are larger than the other ones in size, taking the form of a criss-cross or modification of it. They may correspond with the metacentric V-shaped chromosomes of the spermatogonial cell. The other 50 bivalents take the shape common for those of Decapoda : dumbbell and rectangle. All the bivalents divide synchronously into two equal daughter halves at anaphase.

The symmetrical distribution of the bivalents in the first division gives rise to only one kind of secondary spermatocytes in respect to the chromosome complex. The individual chromosomes of the second metaphase exhibit a distinct dual nature and are scattered all over the plate without any overlapping of chromosomes. Fig. 92 is an example of metaphase equatorial plates. Counting shows that the number of dyads is 52. Nothing particular is found in the mode of the present division ; every dyad divides into two equal daughter monads.

It is concluded that the chromosome number of the present species is 104 in diploid and 52 in haploid. The complex is shown by  $4V' + 100R'$ . There are no chromosomes particular in form and behaviour during meiotic divisions.

#### 20) *Pachygrapsus crassipes* RANDALL

This is also a crab commonly found on rocky sea-shores along the coasts of Japan from Hakodate, Hokkaido to Kyûsyû, having carapace 33.5 m.m. in breadth. It is a member of the Subfamily Grapsinae, Grapsidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. The crabs with which the present study was carried out were captured near the Mitsui Institute of Marine Biology at Suzaki near Shimoda, Izu Peninsula in July, 1936.

The spermatogonia do not undergo simultaneous division, being found intermingled with resting cells. At metaphase the chromosomes constitute a circular equatorial plate showing no overlapping of chromosomes. An example is shown in Fig. 93. After careful counting of many equatorial plates of spermatogonia, it was concluded that the diploid number of chromosomes is 118. The diploid chromosomes complex is of isomorphic nature : all the chromosomes are acrocentric in attachment and vary in length from short rod to minute dot. It seems impossible to identify the homologous pairs, because they diminish gradually in size with slight differences.

Since the division of primary spermatocytes takes place synchronously within one cyst, many dividing figures were found in favorable condition for study of the chromosomes. Figs. 94-96 are examples of metaphase polar views of the primary spermatocytes. The dimensions of the equatorial plate are nearly similar to those of *Atergatis floridus*.

There are 58 bivalent distributed evenly in all parts of the plate. Size differences are not very remarkable among them. Every bivalent exhibits a median transverse suture in accordance with the cases previously observed in different kinds of Decapoda : e.g., *Cambaroides japonicus*, *Paralithodes camtschatica*, *Panulirus japonicus*, etc. Closer examination made it clear that every individual complex of the chromosomes involves two more elements in addition to the 58 bivalents mentioned above. The additional two elements do not lie at the same level with the 58 bivalents, therefore being entirely invisible so long as attention is directed to that level. They are first evident only when the focus is changed gradually upward or downward from where the bivalents lie, since they take their position separately on the two 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*. 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 three examples are reproduced (Figs. 97-99). As is readily recognizable from these figures, the two chromosomes in question are not bivalents. They never appear as dumbbell or rectangular in form, but are evidently elements of rod-type, univalent in nature. They differ from each other in their relative magnitude, one being approximately  $2/3$  as long as the other one ; this ratio seems to be invariable. They lie in the central position of the plate, lying probably on a line connecting the two poles of the spindle. At the confronting end they become gradually tapered and more or less pointed at tips, while the opposite ends are rounded. In all the metaphase plates observed, these two distinct chromosomes are situated always considerably off the equatorial plate without exception. Such a structure and situation strongly support the view that these two distinct chromosomes might have been connected distantly together at the pointed ends to establish a distance pairing, as occurred in the sex-trivalent found in *Cervimunida princeps*. At anaphase, they separate from each other and migrate to each pole of the spindle with the daughter halves of ordinary bivalents.

From the facts stated above, these two particular chromosomes are neither bivalents displaced by some mechanical causes, nor chromatoid bodies, but the sex-chromosomes consisting of X and Y, in distance pairing, the X being represented by the larger one while the Y by the shorter one, in likeness to those commonly found in other kinds of animals. For example, the X and Y chromosomes in distance pairing were reported in a male Neuropteran, *Plethosmylus decoratus* by Hirai ('56). Oguma and Asana ('32), Kichijo ('34) and Naville et de Beaumont ('33) also found in some Neuropteran insects the X-Y chromosomes of a similar nature, though they indicated that the situation is the result of precession of the two elements. Based on the above results it is evident that the present species possesses an X-Y sex-determining mechanism in the male. Thus the chromosome constitution of the primary spermatocyte is represented by  $58+XY=59$  ; this is the actual haploid number occurring in the present species.

In the metaphase polar view of the secondary spermatocyte, chromosomes of dual

nature constitute a fairly circular equatorial plate distributing themselves well apart from one another. An example is shown in Fig. 100. There are 59 chromosomes in every equatorial plate under study. There should be present two kinds of secondary spermatocytes, of which one contains the X chromosome and the other involves the Y. But, the distinction of the two particular elements is practically impossible, since the sex-chromosomes have no characteristic shape nor dimension for distinguishment from the autosomes. Further, at anaphase, all dyads including X and Y chromosomes separate synchronously into two equal monads.

Based on the results of the above study, it is evident that the diploid number is 118 in the male of the present species, and that the primary spermatocyte shows 58 bivalents and an X-Y bivalent. Every secondary spermatocyte possesses 59 dyads. The XY sex-mechanism as found in the present species is a new type of sex-determination in the Crustacea, in contrast to the previously described two types:  $X_1X_2$ -Y type in *Cervimunida princeps* and X-O type in *Ovalipes punctatus*.

#### 21) *Hemigrapsus sanguineus* (DE HAAN)

The present species is a common sea-shore crab having a brown carapace about 28m.m. in breadth. It is distinguished from the following species, *Hemigrapsus penicillatus*, since it has yellow strips on the legs and reddish spots on the chelae. It is distributed widely along the coasts of all Japan. It belongs to the Subfamily Varuninae, Grapsidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. Together with *Pachygrapsus crassipes* the present species belongs to the same Family Grapsidae, but to different subfamilies; the present species is a member of the Varuninae while *Pachygrapsus* belongs to the Grapsinae. The materials for the present study were obtained from some specimens captured along the sea-shore in the vicinity of Oshoro near Otaru, in September, 1937.

Many dividing figures of spermatogonia could be observed scattered amongst cells in the resting stage. The chromosomes constitute a circular metaphase plate arranging themselves evenly apart from each other (Figs. 101-102). By careful counting of many excellent metaphase plates, it was decided that the diploid number of chromosomes is 128, all having acrocentric fibre attachment. Since they gradually diminish in size from short rod to dot shape, it was rather impossible to identify the homologous pairs among them.

All the primary spermatocytes within a cyst divide rather synchronously. The dimensions of the metaphase plate are nearly similar in size with those of *Pachygrapsus crassipes*. As seen in Figs. 103-104, there are in the metaphase polar view 63 bivalent chromosomes which were distributed evenly throughout the whole area of the plate. Size-difference exists in a slight degree among them. They all have a dumbbell shape, but the median transverse sutures are observable only in those of the larger size. As was found in the previous species, *Pachygrapsus crassipes*, the present species is also characterized by the presence of two particular chromosomes. Each one of them lies on two sides of the

metaphase plate maintaining a definite distance from the latter. It is possible to catch their images only when the foci are changed from the equatorial plate. They take the central position in the metaphase plate and show a marked difference in their relative sizes. The smaller element is about one-third the larger one in length. The magnitude of the Y chromosome seems to be comparable to that of the smallest chromosome in the spermatogonial garniture (Figs. 103-104). The X is a slender rod-shaped chromosome, tapering at one end toward the Y. The distance between these two chromosomes is remarkably larger than in the preceding species, as seen in the metaphase side view (Figs. 105-106). These particular chromosomes are adequately explicable only by considering them as an X-Y complex in a distance pairing. At anaphase, they separate from each other and migrate to opposite poles together with daughter halves of ordinary bivalents. On this basis, the chromosome formula is formulated by  $63+XY=64$ , representing the haploid number of the present species.

Every equatorial plate of the secondary spermatocyte consists of 64 chromosomes of dyad nature, much reduced in size (Figs. 107-108). There are presumably present two kinds of chromosome complex with regard to the kind of sex-chromosome, irrespective of the same number of chromosomes. In other words, two kinds of secondary spermatocytes, X-class and Y-class, should be produced. But the distinction of these two kinds is impossible, so long as the X- and the Y-chromosomes are difficult of differentiation from others.

It is concluded from the above observation that the present species has 128 chromosomes of rod-type in diploid, and that the chromosome constitution of the primary spermatocyte is represented by  $63+XY=64$  as the male haploid number. The present species is characterized by a distance pairing of the X-Y chromosomes in the male, as was found also in the previous species, *Pachygrapsus crassipes*.

## 22) *Hemigrapsus penicillatus* (DE HAAN)

The present species bears a close resemblance to the previous species, *Hemigrapsus sanguineus* in the general morphology, but is easily distinguishable from the latter by the following three points: 1) having tufts of long brown hairs on its chelae in the male, 2) no reddish spots on its chelae, and 3) no yellow strips on its legs. It belongs also to the Subfamily Varuninae of the Family Grapsidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. It is distributed along the coasts of all Japan. The specimens for this study were captured in shallow sea-water not far from the Mitsui Institute of Marine Biology at Suzaki, near Shimoda, Izu Peninsula, in March 1936.

Divisions of spermatogonia do not occur simultaneously within a cyst, dividing cells being found intermingled with the resting cells. Two kinds of spermatogonia, the early and late, may be classified only by their sizes. Fig. 109 is an example of an excellent spermatogonia in which chromosomes arrange themselves well apart from one another without overlapping. The counting of chromosomes, therefore, was very easy in spite of their large number; it showed that the spermatogonial complex consists of 138 chromo-

somes. The diploid garniture is of polymorphic nature ; all the chromosomes are acrocentric rod-shaped, except only one pair, each of which is metacentric V-shaped. They show submedian attachment. There is difficulty in the identification of the homologous pairs in the acrocentric elements except for the longest ones which are remarkable by having slightly curved shape.

Dividing figures of primary spermatocytes could be observed abundantly within a cyst. Every metaphase equatorial plate contains 68 bivalents of dumbbell and rectangular shape (Figs. 110–111). In a few of the larger bivalents, median transverse sutures are distinct. Careful observation of many metaphase plates in both polar and side views made it clear that the present species is also characterized by an X and Y sex-mechanism as was found in the previous two species, *Pachygrapsus crassipes* and *Hemigrapsus sanguineus*. As seen in Figs. 112 to 114, the sex-chromosomes in distance pairing take their position at nearly the central position of the equatorial plate lying considerably off from the latter. The distance between the X and Y chromosomes is somewhat shorter than in *Hemigrapsus sanguineus*. The magnitude of the Y chromosome seems to be comparable to that of the third, or fourth smallest chromosome in the spermatogonial garniture. The Y chromosome is about one half the X chromosome in magnitude. The X chromosome is not slender but rather plump, tapering at one end toward the Y. At anaphase, the X element separates from the Y and they migrate to each pole with daughter halves of ordinary bivalents. The chromosome formula of the primary spermatocyte is therefore represented by  $68+XY=69$  that is the haploid number of the present species.

Dividing figures are rather scanty in the secondary spermatocyte. They are found near the cysts of primary spermatocytes in process of division. There are 59 chromosomes having distinct dyad structure in every metaphase plate (Fig. 115). It was actually impossible to distinguish two kinds of chromosome complex of secondary spermatocytes in respect to sex-chromosomes, because there are many chromosomes having nearly equal size which makes difficult the identification of the X and the Y chromosomes in the garniture.

Based on the above results the conclusion was possible that the present species is characterized by 138 chromosomes in the diploid complex which shows  $2V'+136R'$ , and that the primary spermatocyte complex is shown by  $68+XY=69$ . The XY complex is remarkable by showing a distance pairing at the first metaphase, as occurred likewise in two species of the Family Grapsidae.

### 23) *Eriocheir japonicus* DE HAAN

The present species is a common crab distributed all over Japan as an inhabitant of fresh or brackish waters in rivers, streams or river mouths. It is characterized by carapace 62 m.m. in breadth and long brown tufts of hairs on both its chelae. It is also a member of the Subfamily Varuninae together with the preceding two species of *Hemigrapsus*. The crabs from which the material of the present work was secured, were

collected from shores near the Mitsui Institute of Marine Biology, Suzaki near Shimoda, Izu Peninsula in May, 1936.

Dividing figures of spermatogonia are found along the walls of cysts. In the metaphase polar view, the chromosomes appear very clearly as shown in Figs. 116 and 117. By their sizes, the early and late spermatogonia may be distinguishable (Figs. 116 early spermatogonium; Fig. 117, late spermatogonium). In every equatorial plate examined, 148 chromosomes are invariably counted, showing the diploid number of the present species. The spermatogonial garniture is isomorphic in nature: all the chromosomes assume short rod-shape or spherular form having the acrocentric attachment of the spindle fibres. They vary in size slightly. Accordingly, it is vain to attempt to find the homologous pairs among the complex by means of comparison of their sizes.

The dividing figures of the primary spermatocyte could be observed abundantly in a certain cyst. The equatorial plate in diameter shows similar dimensions to the two species of *Hemigrapsus*. An equatorial plate is composed of 73 bivalents distributed evenly throughout the whole area of the plate, as shown in Figs. 118 to 119. The size difference is developed in small degree among these bivalents as in the case of two species of *Hemigrapsus* but the median transverse sutures are also observable in those of bivalents comparatively larger size. It is worthy of notice that in this species, the two peculiar chromosomes are to be discovered again in both sides of the metaphase plate maintaining definite distance from the latter. 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 which tapering at one end toward the smaller one. The distance between the two particular chromosomes is less than in the former two species of *Hemigrapsus*, and nearly identical with *Pachygrapsus crassipes*, a fact which the side view of metaphase plates obviously shows (Figs 120-121). These particular chromosomes in question should also be properly considered as the X and Y showing a distance pairing. The magnitude of the Y chromosome is comparable to those of the smallest or nearly the smallest chromosomes in the spermatogonial garniture. The X-element separates from the Y at anaphase and they migrate to each pole. It is evident from the above observations that the chromosome formula of the primary spermatocyte is represented by  $73+XY=74$ , which is the haploid number of the present species.

Every equatorial plate of the secondary spermatocytes shows 74 chromosomes of dyad nature, quite small in size (Fig. 122). There are presumably present two kinds of secondary spermatocytes with regard to in view of the kinds of the sex-chromosomes. But the distinction of two kinds is absolutely impossible, so long as the identification of the X and the Y chromosomes remains in obscurity.

The results of the present observations of this species are summarized thus: the number of chromosomes is 148 in diploid and 74 in haploid in the male, and the present

species is also characterized by the presence of the XY-mechanism. It is interesting to note that the XY-mechanism was found very clearly in several species of the Family Grapsidae.

#### 24) *Gaetice depressus* (DE HAAN)

The present species is a common small sea-shore crab, common along the Pacific coast of Japan from Muroran, Hokkaido to Kyûsyû. It has a flat carapace of 25 m.m. breadth. It is also a member of the Subfamily Varuninae of the Family Grapsidae with the preceding three species. The material consists of the testes from four specimens collected from shallow sea-water near the Mitsui Institute of Marine Biology, at Suzaki near Shimoda, Izu Peninsula in April, 1937.

As the spermatogonial cells are very scanty in the testes, dividing figures also are very rare. A few polar views at metaphase could fortunately be obtained. Fig. 124 is an example of them. After careful counting of the plate, it was decided that the spermatogonial complex contains 152 chromosomes; 12 are metacentric V-shaped showing 6 homologous pairs, while the remaining 140 are all acrocentric rod-shaped ranging gradually in sizes from long rod to minute dot. The spermatogonial garniture is therefore polymorphic in nature.

In contrast to the spermatogonial cells, primary and secondary spermatocytes are found abundantly in the testes. Since the divisions took place synchronously in those contained within a cyst, many metaphase equatorial plates of primary and secondary spermatocytes could be observed. Figs. 125 and 126 are equatorial plates of primary spermatocytes at metaphase. The dimensions of the plate are nearly equal in diameter with those of the preceding species, *Eriocheir japonicus*. In the metaphase polar view 75 bivalents of dumbbell and rectangular shape distribute themselves equally well apart from one another to constitute a beautiful circular equatorial plate. In some of the larger bivalents appears a median transverse suture. By changing the focus of the microscope gently upward or downward from the equatorial plate where the bivalents lie, one may see two univalent chromosomes differing in sizes from one another. These two particular chromosomes may be considered as X and Y sex-chromosomes. It is of particular importance that the X and the Y are found constantly in the same condition at metaphase being considerably apart from the equatorial plate, lying nearly in the central portion of the equator (Figs. 127-128). The Y chromosome is about 1/2 the X in length. The magnitude of the Y chromosome seems to be comparable to that of the sixth or seventh grade larger than the very smallest size in the spermatogonial garniture. The X chromosome is rather plump in form, tapering at the one of the two ends toward the Y. The distance between the X and the Y is nearly identical to that in *Hemigrapsus sanguineus*. At anaphase, the two separate from each other and migrate to each pole of the spindle with daughter halves of ordinary bivalents. The chromosome formula of the primary spermatocyte is therefore represented by  $75+XY=76$ , as the haploid complex of the present species.

As easily expected from the mode of division in the primary spermatocyte, there should be two kinds of secondary spermatocytes: one containing the X, and the other the Y. As a matter of fact, however, the distinction of these two kinds was not successful because there are many chromosomes having nearly equal sizes to the X and the Y. In the metaphase polar view of secondary spermatocytes, the chromosomes having dyad nature constitute a circular equatorial plate distributing themselves evenly well apart from one another (Fig. 129). There are 76 dyads in every equatorial plate studied. Among them, several elements were observed as having x-shape. They are obviously the descendants of the V-shaped chromosomes in the spermatogonial garniture. At anaphase they separate into two equal monads simultaneously.

The conclusion was reached from the above investigation that the diploid complex of the present species contains 172 chromosomes showing  $12V' + 160R'$ , and that the haploid number is 76 involving an XY sex-mechanism in the male. The fact is remarkably that the studied species of the Family Grapsidae possess the XY sex-mechanism without exception.

#### 25) *Sesarma intermedia* (DE HAAN)

The present species is a half-terrestrial crab, dwelling in swamps and wet places near the sea coast. It measures 32 m.m. in carapace breadth and is distributed widely along the coasts of Asia from Tokyo Bay to the Indo-Pacific region. The crab is a member of the Family Grapsidae, belonging to the Subfamily Sesarminae. The crabs, from which the materials for the present work was obtained, were collected from the bank of a small stream running near the Mitsui Institute of Marine Biology, at Suzaki near Shimoda, Izu Peninsula at various times from March to July 1935. Though preparations were made in large number with these materials, there were found no dividing figures of spermatogenesis except only one which is shown in Fig. 123. In all testes observed, cysts are surrounded with thin layers of spermatogonia in resting stage and the central portions of cysts are almost empty or contain only a small number of mature spermatozoa.

The spermatogonial metaphase plate contains 102 chromosomes having acrocentric structure. Since these chromosomes diminish gradually in size from rod to minute dot, it seems impossible to identify the homologous pairs. Accordingly, it was difficult to deal with the X and Y chromosomes, although such elements were found to occur in five related species of the Family Grapsidae.

The present observations resulted in determining the chromosome number of the present species as 102 in diploid in the male. The existence of the sex-chromosomes remains in question for this species, since no actual observations have been extended at all to the course of meiosis.

#### 26) *Plagusia dentipes* DE HAAN

The present species is a reddish crab with a carapace about 50 m. m. in breadth. It is commonly found along rocky shores of the southern parts of the Pacific coast of Japan.

It belongs to the Subfamily Plagusiinae of the Family Grapsidae, Oxyrhyncha, Brachygnata, Brachyura, Reptanita of the Order Decapoda. The crabs which furnished the material for the present work were collected from shores near the Mistui Institute of Marine Biology, at Suzaki near Shimode, Izu Peninsula at various times during October 1935 and March 1936.

The spermatogonia of the present species do not undergo a simultaneous division, but dividing cells are encountered intermingled with the resting cells. At metaphase, the chromosomes constitute a nearly round equatorial plate. The early and late spermatogonia can be differentiated according to their size. In Figs. 130 to 132, only early spermatogonia are shown. After careful counting in many equatorial plates of spermatogonia it is ascertained without doubt that the diploid number of chromosomes is 106. The chromosomes of the spermatogonial garniture show isomorphic nature; all chromosomes uniformly have acrocentric 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 only slight difference in size or shape.

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 for 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 which distribute themselves evenly throughout the whole area of the plate (Figs. 133-136). Size differences are noted among them in a rather remarkable degree, and the larger ones at least acquire a median transverse suture similar to the cases previously observed in different kinds of Decapoda; e.g. *Paralithodes camtschatica*, *Cambaroides japonicus*, etc. In this species, the two particular chromosomes, X and Y, are to be discovered also at the two sides of the metaphase plate holding a definite distance from the latter as was observed also in the preceding five species of the Family Grapsidae. They take likewise the central position in the metaphase plate and show a marked difference in their relative size. The smaller one is approximately  $\frac{2}{3}$  as long as the larger one, and this ratio seems to be definite without variation. Sometimes a constriction is observable at the middle portion of the larger one, the X. At the confronting ends they become gradually tapered and more or less pointed at the tips, while the opposite ends are rounded. The magnitude of the Y is comparable to those of tenth or eleventh grade larger than the very smallest size in the spermatogonial garniture. It is of particular importance that the X and the Y chromosomes are found constant in distance from each other from the plate in every cell of the same dividing phases (Figs. 137-140). The X and the Y seem to establish distance pairing during the diakinetik stage. The distance between the X and the Y chromosomes is approximately the same as those of *Gaetice depressus*. At anaphase, the X and the Y separate and migrate of the respective poles of the spindle with daughter halves of the autosomes. Thus it is evident that the chromosome constitution of the primary spermatocyte is represented by  $52+XY=53$ ; this number actually does represent

the haploid number of the present species.

The equatorial plate of the secondary spermatocyte is remarkably reduced in dimensions 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 of dyad structure always count 53 without difficulty (Figs. 141-144). Theoretically speaking, there should be present two kinds of secondary spermatocytes of which one contains the X chromosome and the other involves the Y. But their distinction is practically impossible, for there are considerably many chromosomes with slight difference in dimensions which must include the X and the Y chromosomes.

The conclusion may be drawn that the diploid number of the present species is 106 (all rod), and the haploid number is  $53=52+XY$ . The present species is also remarkable for the presence of XY-type sex-mechanism in a distance pairing, as occurred also in five species of the Family Grapsidae.

## ORDER ISOPODA

### 27) *Megaligia exotica* (ROUX)

The present species belongs to the Family Ligiidae, Oniscoidea of the Order Isopoda. It is one of the very common sea-side isopods found all around Japan. Body length of the species is 30-45 m.m. Specimens captured on rocky sea-side places in the vicinity of Oshoro near Otaru, in June, 1954, provided the material for study.

The testicular lobes containing spermatogonial cells are meagre. Since the division of spermatogonia was not take place simultaneously, the metaphase equatorial plates were very few in number. The diploid number 72 was decided through the observations of several reliable spermatogonial metaphase plates. As seen in Fig. 145, the spermatogonial garniture is of polymorphic nature. There are six pairs of metacentric V-shaped chromosomes and 30 pairs of acrocentric chromosomes of rod- and dot-like form in the spermatogonial complex.

The dividing figures of the primary and secondary spermtocytes were abundant in a certain testicular lobe. Every equatorial plate of the primary spermatocytes shows 36 bivalents which scatter equidistantly from one another throughout the whole area of the plate (Figs. 146-147). Size differences are noted among them in a rather remarkable degree; a few larger ones exhibit dumbbell shape, while the remaining smaller ones are rectangular in shape.

The secondary spermatocyte contains at metaphase 98 chromosomes of dyad nature, being much reduced in size as compared with primary spermatocyte (Fig. 148). Several comparatively larger dyads are noticed in the complex: they surely correspond to the V-shaped chromosomes observed in the spermatogonial complex. All the elements divide synchronously at anaphase of both first and second divisions, without showing any element of unusual behaviour.

The chromosome number of the present species, is, therefore, 72 ( $12V'+60R'$ ) in diploid and 36 in haploid in the male.

**28) *Tylos granulata* MIERS**

The present species, together with the preceding one, *Megaligia exotica*, is a member of the Suborder Oniscoidea, but differ in the family. It belongs to the Family Tyliidae, Oniscoidea of the Order Isopoda. It is a terrestrial isopod, being 10 m.m. in body length. It abounds in sandy sea-shores throughout Japan. The present study was based on the testicular material derived from several specimens captured in Ranshima, near Otaru in June, 1950.

A considerable number of dividing figures of spermatogonia could be observed among the spermatogonial cells in the resting stage in one testicular lobe. In the polar view, the chromosomes constitute a fairly rosette shaped equatorial plate, showing the larger chromosomes in the peripheral portion surrounding the smaller ones in central portion. As seen in Fig. 149, the spermatogonial garniture is polymorphic in nature. It was found after careful examination of several spermatogonial metaphases that 68 chromosomes constitute the male diploid complex of this species. Of these 68 chromosomes, 10 pairs are metacentric V-shaped elements and the remaining 24 pairs are acrocentric ones diminishing in size from long rod-shape to minute spherules.

Both first and second divisions of the spermatocytes occur simultaneously and abundantly in another testicular lobe. The chromosomes of the primary spermatocytes are observed with absolute clearness in the metaphase polar view, as they scatter evenly well apart from each other (Figs. 150-151). Every metaphase plate showed 34 bivalents with indisputable clearness. Size differences are rather remarkable: several larger bivalents show the form of a dumbbell with a transverse suture in middle region of each, while the remaining bivalents appear rectangular in form.

In the second metaphase the individual chromosomes exhibit a distinct dual nature; they are scattered all over the equatorial plate without any overlapping. Every metaphase plate examined shows 34 dyads without exception (Fig. 152). One dyad among them is metacentric in nature appearing as x shape. This dyad certainly corresponds to the largest V-shaped chromosome found in the spermatogonial garniture. No chromosome, peculiar in shape or behaviour, was found in either of the meiotic divisions.

To conclude, it is evident that the chromosome number of the present species is 68 in diploid and 34 in haploid in the male, and that the diploid complex is formulated as  $20V'+48R'$ .

**29) *Cleantiella isops* (GRUBE)**

The present species is a member of the Family Idoteidae, Valvifera of the Order Isopoda. It is distributed widely along the coasts throughout Japan. It measures 20 to 30 m.m. in body length. The specimens obtained in the vicinity of the Toyoura Marine Biological Station, near Hakodate, in August, 1957, furnished the material for study.

Three pairs of testicular lobes of a testis do not show the same stage in process of maturation. The anterior lobe contains spermatogonial cells, the middle lobe is provided with spermatocyte of different stages, while the posterior lobe is filled with mature spermatozoa.

As occurred in the case of Decapoda, in the present species of Isopoda the spermatogonia do not undergo simultaneous division in a cyst, but dividing figures are encountered independently scattered among the cells in the resting stage. Several countings were made of the chromosomes in dividing spermatogonia. Every metaphase plate constantly showed 64 chromosomes as diploid number. An example is shown in Fig. 153. As seen in the figure, the spermatogonial garniture is polymorphic in nature; of 64 chromosomes, 34 elements are metacentric V-shaped and the remaining 30 are acrocentric rod- and dot-shaped elements. All the elements can be paired in the diploid complex.

Division of the spermatocyte took place in almost all the cells in a testicular lobe excepting cells at both terminal portions of the lobe which are in the stages of meiotic prophase. Many dividing figures, therefore, were observed in the primary and secondary spermatocytes; the bivalents take their appearance with distinct clearness, scattering evenly on the equatorial plate, as drawn in Fig. 199. In the present stage, therefore, the number of chromosomes can be counted with much ease and without error. There are observable 32 bivalents in every metaphase plate of the primary spermatocyte. About a half of the number of bivalents take the form of criss-cross tetrad or its modified forms. It is certain that these bivalents are the descendants of 17 pairs of V-shaped chromosomes in the spermatogonium. The remaining bivalents exhibit dumbbell and rectangular forms. Even in larger dumbbell shaped bivalents the transverse suture at the middle region could not be seen.

The metaphase polar view of the secondary spermatocyte shows 32 chromosomes of dyad nature (Fig. 155). All the the chromosomes corresponding to those of the spermatogonium are to be pointed out too in the garniture of the secondary spermatocyte: 17 metacentric V-shaped chromosomes and the remaining acrocentric ones varying from rod to dot shape.

The chromosome number of the present species is therefore 64 (34V' + 30R') in diploid and 32 in haploid. There are no chromosomes which show form and behaviour characteristic to the sex-chromosomes during two meiotic divisions.

### 30) *Idotea japonica* RICHARDSON

The present species is also a member of the Family Idoteidae, Valvifera, of the Order Isopoda: individuals are 25–45 m.m. in body length. They are very common sea-shore animals in northern parts of Japan. The specimens for the present study were collected abundantly in the same place and simultaneously with the preceding species, *Cleantiella isops*.

The constitution and condition of the testicular lobes of the present species, are quite similar to those of the preceding species. The dividing figures of spermatogonia are found

here and there among the spermatogonial cells in the resting stage independently of the neighbouring cells. In the polar view the chromosomes assume a rosette form in their metaphase arrangement, as the larger ones occupy the peripheral position in the equatorial plate surrounding the smaller ones in the central part. In order to determine the number and form of chromosomes, many equatorial plates were examined in their polar aspect. Examples are given in Fig. 156. The diploid number observed in the spermatogonium was decided as 64. As is recognizable in the figure, the spermatogonial garniture is of polymorphic structure; of the 64 chromosomes, 30 are metacentric elements while the remaining 34 are all acrocentric varying in shape from long rod to short rod. According to their sizes and shapes all the chromosomes can be arranged into 32 homologous pairs. One pair amongst the metacentric chromosomes are satellite-like in structure, since each chromosome consists of a small dot-like body and a long rod-like one connected thereto by means of a fine thread. The other metacentric elements forming the 14 pairs are of V-shape. They all have nearly submedian attachment of the spindle fibres.

One pair of the three pairs of testicular lobes is filled with cells of primary and secondary spermatocytes in process of division. It is not difficult to find the dividing figures of spermatocytes, in which the chromosomes are seen in favourable condition for study. Viewed from the pole of division of primary spermatocytes, it is very clear that every metaphase plate contains 32 bivalent chromosomes, distributed evenly throughout the whole area of the plate (Fig. 157). All the bivalents take rectangular form indicating no sign of constitutional morphology. At anaphase all the bivalents separate synchronously into two equal halves.

Careful observations of a considerable number of the metaphase plates of the secondary spermatocytes have revealed that the primary spermatocyte division actually gives rise to only one kind of secondary spermatocytes with respect to the complex of chromosomes. The chromosomes are generally much more slender in form, compared with that of the primary spermatocyte. Careful counting shows, without any exception, 32 dyads in every metaphase equatorial plate under study. Fig. 158 is an illustration of them. As seen in the figure, they all exhibit dyad nature. There are several dyads which evidently show by their shape that they are the descendants of V-shaped chromosome found in spermatogonial garniture. No chromosome having any peculiar form and behaviour could be detected throughout the two reduction divisions.

In summary, the chromosome number of the present species is 64 ( $30V' + 34R'$ ) in diploid and 32 in haploid. The species is identical in chromosome number to the previously described one, *Cleantiella isops*, but is different in chromosome constitution; namely, the previous species has four more metacentric chromosomes and four less acrocentric ones than the present one. The presence of a pair of satellite-like chromosomes is also a characteristic feature peculiar to the present species.

### 31) *Cymodoce japonicus* RICHARDSON

The present species belongs to the Subfamily Sphaerominae, Sphaeromidae, Flabellifera of the Order Isopoda. It is 10–25 m.m. in body length. It dwells in the rocky sea-shore along the coasts of Japan from Hokkaido to Kyûsyû. The specimens for this study were collected from holes in wooden piers made by the shipworm, *Teredo*, along the sea-shore of the Akkeshi Marine Biological Station, in September, 1944.

The testes of the present species, consisting of three pairs of testicular lobes, are similar in structure with those of the previously described four isopods. The condition of each testicular lobes which show different stages of meiotic process in each, is also quite comparable to that of the preceding four isopods, *Megaligia exotica*, *Tylos granulata*, *Cleantiella isops* and *Idotea japonicus*.

Dividing figures of spermatogonia were observed abundantly within a lobe. The chromosome arrange themselves well apart from one another, forming a fairly circular equatorial plate. Careful counting of excellent spermatogonial metaphase plates gave the diploid number of 58 without even slight doubt. As seen in Fig. 159, the spermatogonial garniture is of polymorphic nature: of 58 chromosomes, 32 are metacentric elements of V-shape and 26 are acrocentric ones of rod- or dot-shape. All the chromosomes of the spermatogonium can be arranged into to 29 homologous pairs.

In the metaphase polar view of the primary spermatocyte the bivalents take their appearance with distinct clearness, scattered evenly on the equatorial plate, as drawn in Figs. 160 and 161. In the present stage, therefore, the number of chromosomes can be counted with much ease and without mistake. The primary spermatocyte contains 29 bivalents without exception. There are several bivalents which are considered to be ring-tetrads. They evidently correspond to the larger metacentric V-shaped chromosomes in the spermatogonium. The remaining bivalents exhibit a dumbbell or rectangular shape. No transverse suture across the middle region of each dumbbell-shaped bivalent, though it is rather common in the bivalents of Decapoda, could be seen in the present species. At anaphase, all the bivalents separate into two equal halves and migrate synchronously to the poles of the spindle. No chromosome, peculiar in shape and behaviour, was found in the present division.

As obvious from the mode of division of the first spermatocyte, there is only one kind of secondary spermatocytes concerned in the chromosome complex. In the polar view of the second spermatocyte at metaphase, 29 chromosomes of dyad structure constitute a fairly circular equatorial plate (Fig. 162). Among these dyads, several appear as metacentric ones. They may be the descendants of larger V-shaped chromosomes in the spermatogonial garniture. At anaphase, nothing particular is found in the mode of the secondary spermatocyte division; every dyad divides into two equal daughter monads.

It may then be summarized that the chromosome number of the present species is 58 in diploid and 29 in haploid in the male, and that the diploid complex is represented by  $32V' + 26R'$ . As described above, the present species throughout the two reduction divisions

contains no particular chromosome regarded as the sex-determining element.

### 32) *Tecticeps japonicus* IWASA

The present species is one of the marine isopods belonging to the Subfamily Sphaerominae, Sphaeromidae, Flabellifera of the Order Isopoda. It measures 10–15 m.m. in body length. They are common around the sea-shores of the Hokkaido and the Kurile Islands. The material upon which the present study was based was obtained from animals collected in the vicinity of the Akkeshi Marine Biological Station, Hokkaido in June and July in the years, 1950 to 1954.

The testes of the present species consist of three pairs of testicular lobes, each having a spindle shape, as in the preceding five species. The terminal parts of the testicular lobes are occupied by spermatogonial cells and the other parts are filled with cells at various stages of meiosis. The dividing figures of spermatogonial cells are not frequent, by found the metaphase figures being rather rare. Examples of ideal metaphase plates are shown in Fig. 163 and 164. After careful counting of the chromosomes in several such plates, it was found that the diploid number was invariably 63. It is quite natural to expect the existence of a single X-chromosome based on the occurrence of the odd chromosome number in the spermatogonial division. The spermatogonial chromosomes are polymorphic in the complex; the complex consists of a certain number of acrocentric and metacentric chromosomes. As seen in Fig. 165 which is a serial alignment of the supposed pairs of chromosomes, there are 18 pairs of acrocentric chromosomes of rod-type, 13 pairs of metacentric of V- and J-shape, and a single, large metacentric element. The latter is very conspicuous among the others since it is the largest of all in size, submedian in fibre attachment, and unpaired having no homologous mate. This finding is explicable only by assuming this element to be the X-chromosome.

The majority of germ cells are found in process of meiosis in the testicular lobes fixed in June and July. The first and second divisions were available for study in the material collected in approximately the first week of July.

The primary spermatocytes in the metaphase side view clearly and invariably show remarkable precocious migration of a V-shaped chromosome of outstandingly large size, in striking contrast to the autosomal bivalents which lie in the equatorial plate. This element which makes the precocious migration to one of the poles is in all probability the X-chromosome because of its characteristic feature as seen in the former division and of its particular behaviour like the X of many animals (Figs. 170–171). In the early anaphase stage (Fig. 172) this particular element reaches one of the poles, while the autosomal elements are on the way thereto. Apparently it is univalent in structure.

The number of bivalents observed in the equatorial plate is 31 without exception. In addition to these autosomal bivalents, the X-chromosome makes its appearance when the focus of the microscope is changed a little upward or downward from the equatorial plate, being very conspicuous for its large V-shaped, univalent configuration (Figs. 167–

168). The majority of the bivalents take the form of a criss-cross tetrad, while the remaining ones are dumbbell and rectangular in form. These bivalents in criss-cross shape are surely the descendants of the metacentric V-shaped chromosomes in the spermatogonial garniture. In the first meiotic division the X-element moves to one of the poles without division, resulting in the production of two kinds of secondary spermatocytes; one having the X and the other containing no such one.

Observations of the diakinetin nucleus revealed that one large element has remained in a heteropycnotic condensation showing a distinct V-shape. This is no other than the X-element (Fig. 166) which corresponds to such an element observed in the first meiotic division.

It is thus evident that the X-chromosome of this form is characterized by a distinguishable V-shape and large size, remarkable precocious separation in the first meiotic division and heteropycnosis in the growing stage.

The chromosome constitution of the primary spermatocyte is shown by the formula,  $32=31+X$ , as the haploid number of the present species in the male.

As the result of the first division, it is evident that there are to be produced two kinds of secondary spermatocytes, the X-class cell containing the X-element and no-X-class cell. The present material furnished a few dividing secondary spermatocytes in which only an X-class cell was observed (Fig. 169). The equatorial plate of the secondary spermatocyte is remarkably reduced in dimensions as well as the chromosomes themselves compared with the size of the primary spermatocyte, although they have strong resemblance in appearance to the latter. The equatorial plate contains 31 dyads and a large V-shaped chromosome in addition which is the X-element.

On the basis of the above facts, it is concluded that the present species shows male heterogamety represented by an X-O mechanism. This finding is quite unique in the Order Isopoda, so far as the chromosomal survey has gone.

## ORDER AMPHIPODA

### 33) *Anisogammarus annandalei* (TATTERSALL)

The present species is a common one of fresh water amphipods in Hokkaido. It belongs to the Family Gammaridae, Gammaridea of the Order Amphipoda. It measures 15 to 20 m.m. in body length. The specimens from which the material was derived were collected in July 1934 in a small stream running through the Hokkaido University campus.

Since the divisions of the spermatogonia are not simultaneous in the testicular cyst, the metaphase figures are not abundant. After careful counting of the chromosomes, the diploid number was determined to be constantly 54 in the spermatogonial complex; all the elements are uniformly acrocentric in their fibre attachment. They range from rods of medium size to those dot-like in shape. It is, therefore, impossible to pair them by a

comparison of their size and shape. The even number of chromosomes suggests the occurrence of an XY-pair. Careful observations reveals the existence of a chromosome which is characterized by its small size. It is solitary having no homologous mate in the complex and is about two-thirds the size of the elements of the smallest pair of chromosomes. Because of its unpaired condition and of its smallest size, this element is probably the Y chromosome (*y* in Figs. 173-174). It is difficult to identify the X chromosome in the diploid complex.

The resting nucleus of the primary spermatocyte contains two very conspicuous chromatin nucleoli (Figs. 179-186, *xy*). They are easily distinguished from the plasmosome nucleolus by the intense affinity for haematoxylin and by the peculiar form as well. Further, one of them is smaller, having a size one-third that of the other. Through all stages of the growth period, these two nucleoli are connected with each other by a fine chromatin thread. In the leptotene stage, the chromatin thread is considerably elongated, having two nucleoli at each end (Figs. 179-182). The association between the plasmosome and the chromatin nucleoli does not occur through the growth period. At the commencement of diakinesis, the chromatin thread becomes shortened and the larger nucleolus assumes an angular shape (Figs. 183-186). Under this condition, the difference between the two elements of the nucleoli becomes very evident.

At metaphase of the first division, these two elements forming the nucleoli, lie in the equatorial plate assuming an end-to-end conjugation with their long axes at right angles to the plate (Fig. 187). At anaphase, they separate and migrate to the opposite poles. Judging from such a behaviour, these two unequal elements seem to be no other than the sex-chromosome of the present species, the larger being the X-chromosome and the smaller the Y-element.

In the polar view of the first division, 27 chromosome are arranged with distinct clearness, as shown in Figs. 175-178. In the side-view (Fig. 187), the XY complex is clearly distinguishable from the autosomal bivalents. It is evident from the above facts that the chromosome formula of the primary spermatocyte is  $26+XY=27$ .

Every equatorial plate of the secondary spermatocytes shows 27 chromosomes (Figs. 188-191). There are two kinds of secondary spermatocytes, of which one contains the X-chromosome and the other the Y. But the distinction of them is practically impossible, because of the scantiness of distinguishing characters available for identification.

As mentioned above, the present observations establish the existence of the XY-mechanism of sex-chromosomes in male germ cells of this species. The diploid number is 54 and the haploid number is 27.

## V. PART II. CHROMOSOME MORPHOLOGY IN RELATION TO TAXONOMICAL CLASSIFICATION OF THE CRUSTACEA

The taxonomy, variation and distribution of animals have been dealt with by a number of taxonomists. On the other hand, the chromosome cytology has largely contributed to animal systematics in several different ways: cytological data serve as useful and fundamental criteria for the diagnosis of species, and for the understanding of the evolutionary mechanism of organisms, since changes in chromosomes involving various types of inner structural rearrangements play an important role in the formation of species (White '57 a,b). The application of cytological methods to taxonomy has increasingly made possible the solution of discrepant taxonomical matters that are always difficult for non-cytologists to solve. The cytotaxonomic differences which are generally noted by cytologists deal with differences in chromosome number, or in the sizes and shapes of some of the chromosomes in the complex.

In the following pages, the author shall refer to any cytologically detectable differences in related orders, families, genera and species of the Crustacea. Further, consideration will be given to the contribution of the cytology to the taxonomy of some crustaceans, briefly viewing the evolution of three orders, Decapoda, Isopoda and Amphipoda of the Class Crustacea.

Table 1 shows the numerical relationship of the chromosomes in the Decapoda, Isopoda and Amphipoda of the Crustacea so far reported on.

It is evident by reference to Table 1 that, in the Decapoda, the chromosome number shows a wide range of variation from  $2n=82$  in *Gebia major* (Oka '41) to  $2n=254$  in *Eupagurus ochotensis* (Niiyama '44). The Isopoda give rather small counts which vary from  $2n=12$  in *Anilocra mediterranea* (Callan '40) to  $2n=72$  in *Megaligia exotica* (Niiyama '59). In the Amphipoda, on the other hand, the counts are comparatively uniform giving from  $n=23$  in two species of *Maera* to  $n=27$  in *Gammarus duebeni* (Le Calvez et Certain '51), excepting one species, *Marinogammarus pirloti* (Orian and Callan '57) which shows a chromosomal polymorphism such as  $n=29, 30, 31, 32$ .

### 1) Order Decapoda

The chromosome number of the Decapoda shows a considerable variation within the Order (see Table 1). In the Suborder Natantia chromosome counts have been made in only three species, *Crangon cataphractus* (Carnoy '85), *Pandalus borealis* (Leopoldseder '34) and *Penaeus japonicus* (Niiyama '48). The data from the former two species may be doubtful by present standards of cytology, since the chromosome figures presented in their papers seem to be derived from poorly preserved specimens. The counts,  $2n=92$ ,  $n=46$ , in *Penaeus japonicus* is a rather small number among the Decapoda and seems to have closer relationship to the Brachyura than to the other groups of Reptantia of the Decapoda. Definite conclusion, however, must be postponed because there is available only one

Table 1. Chromosome numbers of decapods, isopods and amphipods so far reported

## DECAPODA

Species	2n	n	Author
Suborder Natantia			
Group Penaeidea			
Family Penaeidae			
<i>Penaeus japonicus</i>	92s (14V'+78R')	46 ♂	Niiyama '48
Group Eucyphidea			
Tribe Pandaloidea			
Family Pandalidae			
* <i>Pandalus borealis</i>		34 ♂ I 32+2X ♂ II	Leopoldseder '34
Tribe Crangonida			
Family Crangonidae			
* <i>Crangon cataphractus</i>		40-44 ♂	Carnoy '85
Suborder Reptantia			
Group Palinura			
Family Palinuridae			
<i>Panulirus japonicus</i>	140 (12V'+128R')	70 ♂	Niiyama '36a,b
Group Astacura			
Family Potamobiidae			
* <i>Astacus fluviatilis</i>		ca. 58 ♂	Prowazek '02
<i>Cambarus virilis</i>	200s	100 ♂	Fasten '14
<i>Cambarus immumis</i> (?)		98+8 ♂ I	Fasten '14
<i>Cambarus clarkii</i>	192s (6V'+186R')	96 ♂	Niiyama '34
<i>Cambaroides japonicus</i>	196s (all rod)	98 ♂	Niiyama '34
Family Nephropsidae			
* <i>Homarus</i>		18 ♂	Labbé '40
<i>Nephrops japonicus</i>	164s (all rod)	82 ♂	Niiyama '39
<i>Nephropsis carpenteri</i>	152s (30V'+122R')	76 ♂	Niiyama '39
Group Anomura			
Tribe Thalassinidea			
Family Callinassidae			
<i>Gebia major</i>	82s,o	41 ♂	Oka '41
Tribe Galateidea			
Family Galateidae			
<i>Cervimunida princeps</i>	109s (18V'+91R')	53+XXY ♂ I	Niiyama '59
Tribe Hippidea			
Family Hippidae			
<i>Hippa talpoides</i>		60 ♂	Nichols '09
Tribe Paguridea			
Family Paguridae			
<i>Eupagurus ochotensis</i>	254s (18V'+236R')	127 ♂	Niiyama '44
* <i>Eupagurus prideuxii</i>		12 ♀	Weismann & Ishikawa '88
Family Coenobitidae			
<i>Coenobita rugosa</i>	230s (all rod)		Niiyama This paper
Family Lithodidae			
<i>Paralithodes camtschatica</i>	208s (all ord)	104 ♂	Niiyama '36a,b
<i>Paralithodes platypus</i>	206s (18V'+188R')	103 ♂	Niiyama This paper

Group Brachyura			
Tribe Oxystomata			
Family Carapidae			
<i>Matua lunaris</i>	94s (4V'+90R')	47 ♂	Niiyama '42
Family Leucosiidae			
<i>Philyra pisum</i>	114s (4V'+110R')	57 ♂	Niiyama This paper
Family Raninidae			
<i>Ranina ranina</i>	106s (24V'+82R')	53 ♂	Niiyama '42
Tribe Brachygnata			
Superfamily Oxyrhyncha			
Family Majidae			
<i>Macrocheira kaempferi</i>	106s (16V'+90R')	53 ♂	Niiyama '39
Superfamily Brachyrhyncha			
Family Portunidae			
Subfamily Portuninae			
<i>Ovalipes punctatus</i>	103s (4V'+99R')	51 + X ♂ I	Niiyama '41
Subfamily Carcininae			
* <i>Carcinus maenus</i>		30-40 ♂	Carnoy '85
Subfamily Lupinae			
<i>Scylla serrata</i>	106s (all rod)	53 ♂	Niiyama '42
Family Atelecyclidae			
<i>Telmessus cheiragonus</i>	124s (18V'+116R')	62 ♂	Niiyama '42
Family Cancridae			
<i>Cancer gracilis</i>		52 ♂	Fasten '24
<i>Cancer magister</i>		60 ♂	Fasten '18
<i>Cancer oregonensis</i>		56 ♂	Fasten '24
<i>Cancer productus</i>		58 ♂	Fasten '24
Family Xanthidae			
<i>Atergatis floridus</i>	104s (4V'+100R')	52 ♂	Niiyama This paper
<i>Lophopanopeus bellus</i>		62 ♂	Fasten '26
* <i>Menippe mercenaria</i>	51-80s	25-28 ♀	Binford '13
Family Potamonidae			
<i>Potamon dehaani</i>	82s (about half V' or J')	41 ♂	Yanagita '44
* <i>Telphusa fluviatilis</i>	78s	38 + 2X ♂ II	Delpino '34
Family Grapsidae			
Subfamily Grapsinae			
<i>Pachygrapsus crassipes</i>	118s (all rod)	58 + XY ♂ I	Niiyama This paper
Subfamily Varuninae			
<i>Hemigrapsus sanguineus</i>	128s (all rod)	63 + XY ♂ I	Niiyama '38
<i>Hemigrapsus penicillatus</i>	138s (2V'+136R')	68 + XY ♂ I	Niiyama This Paper
<i>Eriocheir japonicus</i>	148s (all rod)	73 + XY ♂ I	Niiyama '37
<i>Gaetice depressus</i>	152s (12V'+140R')	75 + XY ♂ I	Niiyama This paper
Subfamily Plagusinae			
<i>Plagusia dentipes</i>	106s (all rod)	52 + XY ♂ I	Niiyama '37a,b

## ISOPODA

Suborder Asellota			
Family Asellidae			
* <i>Asellus aquaticus</i>	20-30s	8-10 ♂	Carnoy '85
<i>Asellus aquaticus</i>	16s	8 ♂	Vandel '38 '41
<i>Asellus aquaticus</i>	16s	8 ♂ I	Vitagliano '47
* <i>Asellus aquaticus</i>	17s	7 + X ♂ I	Dworak '35

<i>Asellus (Proasellus) meridianus</i>	16s	8 ♂ I	Vandel '38, '41
<i>Asellus nipponensis</i>	14s	7 ♂ I	Sugiyama '33
<i>Stenasellus virei</i>		27 ♂ I	Vandel '41, '47
Family Janiridae			
<i>Jaera marina forsmanni</i>		8+XYY ♀ I	Steiger & Bocquet '54
<i>Jaera marina ischiosetosa</i>		12+XYY ♀ I	Steiger & Bocquet '54
Suborder Oniscoidea			
Family Oniscidae			
Subfamily Armadillidiinae			
<i>Armadillidium opacum</i>	54s	27 ♂	Radu '30
<i>Armadillidium nasatum</i>		28 ♀ I	Vandel '41
<i>Armadillidium vulgare</i>	54s	27 ♂ I	Radu '31
<i>Armadillidium vulgare</i>		28 ♂ I	Vandel '41
Subfamily Oniscinae			
<i>Oniscus asellus</i>	32s	16 ♂ ♀	Nichols '01, '02' '04
<i>Chaetophiloscia elongata</i>		24 ♀ I	Vandel '41
<i>Philoscia gallicus</i>		24 ♀ I	Vandel '41
Subfamily Porcellioninae			
<i>Porcellio scaber</i>	56s (all V')	28 ♂	Imai & Makino '40
<i>Porcellio scaber</i>		28 ♀ I	Vandel '41
<i>Porcellio gallicus</i>		28 ♀ I	Vandel '41
<i>Porcellio loevis</i>		28 ♀ I	Vandel '41
<i>Porcellio muscorum</i>		24 ♀ I	Vandel '41
Family Ligiidae			
<i>Ligia italica</i>		28 ♂ ♀	Mir '39
<i>Ligia oceanica</i>		30 ♂	Mir '39
<i>Ligidium hypnorum</i>		31 ♂	Mir '39
<i>Megaligia exotica</i>	72s (12V'+60R')	36 ♂	Niiyama '59
Family Tyliidae			
<i>Tylos granulata</i>	68s (20V'+48R')	34 ♂	Niiyama '59
Suborder Valvifera			
Family Idoteidae			
<i>Cleantiella isops</i>	64s (34V'+30R')	32 ♂	Niiyama '59
<i>Idotea irrorata</i>		28 ♂	Nichols '09
<i>Idotea japonica</i>	64s (30V'+34R')	32 ♂	Niiyama '59
Suborder Flabellifera			
Family Cymothoidae			
<i>Anilocra mediterranea</i>	12s, o	6 ♂	Callan '40
Family Sphaeromidae			
<i>Cymodoce japonica</i>	58s (32V'+26R')	29 ♂	Niiyama '59
<i>Tecticeps japonicus</i>	63s (27V'+36R')	31+X ♂ I	Niiyama '56
Suborder Epicaridea			
Family Bopyridae			
* <i>Epipenaeon japonica</i>	16c1	8p. b.	Hiraiwa '36

## AMPHIPODA

Suborder Gammaridea			
Family Gammaridae			
<i>Anisogammarus annandalei</i>	54s (all rod)	26+XY ♂ I	Niiyama '35 '50
<i>Carinogammarus roeseli</i>		26 ♂ I	Le Calvez '49
<i>Echinogammarus bevilloni</i>		26 ♂ I	Le Calvez '49
* <i>Gammarus chevreuxi</i>		ca. 22	Huxley '23
* <i>Gammarus chevreuxi</i>	26s	12+XY ♂ I	Palmer '25, '26
<i>Gammarus chevreuxi</i>	52s	26 ♂ I	Le Calvez et Cer- tain '51
<i>Gammarus chevreuxi</i>		26 ♀ II	Orian & Callan '57

* <i>Gammarus duebeni</i>		13	Le Roux '33
<i>Gammarus duebeni</i>	54s (over 50V')	27 ♂ I	Le Calvez et Certain '51
<i>Gammarus duebeni</i>		27 ♀ II	Orian & Callan '57
<i>Gammarus locusta</i>		26 ♂ I	Poisson et Le Calvez '48
<i>Gammarus locusta</i>		26 ♀ II	Orian & Callan '57
<i>Gammarus marinus</i>	52s	26 ♂ I	Le Calvez et Certain '51
<i>Gammarus pulex pulex</i>	52s	26 ♂ I	Poisson et Le Calvez '51
<i>Gammarus pulex</i>	52, 53cl	26, 27 ♀ II	Orian & Callan '57
<i>Gammarus pungens</i>	48s	24 ♂ I	Le Calvez et Certain '51
<i>Gammarus zaddachi</i>		26 ♂ I	Le Calvez '49
<i>Maera othonis</i>		23 ♂ I	Le Calvez et Certain '51
<i>Maera grossimana</i>		23 ♂ I	Le Calvez et Certain '51
<i>Marinogammarus finmarchus</i>		26 ♀ II	Orian & Callan '57
<i>Marinogammarus longipes</i>		26 ♂ I	Le Calvez '49
<i>Marinogammarus marinus</i>		25, 26 ♀ II	Orian & Callan '57
<i>Marinogammarus obtusatus</i>		26 ♀ II	Orian & Callan '57
<i>Marinogammarus pirloti</i>		29, 30 31, 32 ♀ II	Orian & Callan '57
<i>Niphargus plateau</i> var. <i>elongatus</i>		25 ♂ I	Le Calvez et Certain '51
<i>Niphargus tatrentis</i> var. <i>lunzensis</i>	50s (2V' + 48R')	25 ♂ I	Le Calvez et Certain '51
Family Talitridae			
<i>Orchestia gammarella</i>		25 ♂ I	Poisson et Le Calvez '48
<i>Orchestia mediterranea</i>		25 ♂ I	Le Calvez et Certain '51
<i>Talitrus saltator</i>		25 ♂ I	Poisson et Le Calvez '48
<i>Talorchestia deshayesi</i>		25 ♂ I	Le Calvez et Certain '51
* <i>Talorchestia longicornis</i>		18	Nichols '09

Abbreviations used: - s: spermatogonium; o: oogonium; cl: cleavage; p.b.: polar body; I: primary spermatocyte; II: secondary spermatocyte; V': metacentric chromosome; R': acrocentric chromosome; \*The data marked with asterisks are those unsatisfactory by present-day-standards of cytology.

reliable datum at present.

The Suborder Reptantia is divided taxonomically into four groups, viz., Palinura, Astacura, Anomura and Brachyura. In the group Palinura, only one species, *Panulirus japonicus*, was studied cytologically by the present author (Niiyama '36a, b). It shows 140 in  $2n$  and 70 in  $n$ . The characteristic feature is that this species has compound ring-tetrads together with the ordinary bivalents in the primary spermatocyte: they are the descendants of large V-shaped metacentric chromosomes in the spermatogonial cells. Such bivalents have never been reported to occur in other species of Crustacea so far studied. Accordingly, it is rather difficult to consider the relationship of this species to other groups of Crustacea.

In the Group Astacura, eight species belonging to two families, Potamobiidae and Nephropsidae, were studied cytologically. Setting aside the two suspicious cases of *Astacus fluviatilis*  $n=ca. 58$  (Prowazek '02) and *Homarus*  $n=18$  (Labbé '04), four members

of the Potamobiidae have more or less than 200 chromosomes, while two members of the Nephropsidae are characterized by a smaller number than the former. The Astacura seems to have a close relationship to the Tribe Paguridea of the group Anomura in view of from the chromosome set-up. Two species of the Family Nephropsidae, *Nephrops japonicus* and *Nephropsis carpenteri*, seem to be closely related from the view point of chromosome morphology on the basis of the presence of unusual quadripartite bivalents in each species. Special interest is attracted from the morphological view-point when one considers the evolution of the chromosome complex in Crustacea that in spite of their close taxonomical relation, the former species has 164 chromosomes of rod-shape, while the diploid complex of the latter consists of 30 V-shaped metacentric chromosomes and 122 rod-shaped ones.

In the Group Anomura, the chromosome number varies considerably among the different Tribes. The group is divided into four Tribes: Thalassinidea, Galateidea, Hippidea and Paguridea. In the former three Tribes, the following chromosome numbers have been reported:  $2n=82$  in *Gebia major* (Oka '41),  $2n=109$  in *Cervimunida princeps* (Niiyama '59) and  $n=60$  in *Hippa talpoides* (Nichols '09). Those counts fairly approximate those of the Brachyura Group. Moreover, that *Cervimunida princeps* has an  $X_1X_2Y$ -type sex-mechanism in the male, suggests the occurrence of kinship to the Brachyura in which several species have an XO or XY sex-mechanism in the male, since it has repeatedly been stated that the XX-Y mechanism was secondarily derived from either an XO- or XY-condition by translocation involving an autosome and sex-chromosomes (White '40, '41, '54, Cooper '46, '51, Hughes-Schrader '47, '50). In contrast to the above three Tribes, the Paguridea is characterized by a high number of chromosomes, all the species so far reported having over 200. The highest number in animals so far studied was obtained in *Eupagurus ochotensis* (Niiyama '44) which shows  $2n=254$ . With respect to the number of chromosomes, there is a close relationship between Paguridea and Astacura.

In the Group Brachyura, twenty-three species have been investigated cytologically. All the species have about 100 chromosomes, excepting several species of the Family Grapsidae in which there have hitherto been reported  $2n=152$  in *Gaetice depressus* (Niiyama This paper),  $2n=148$  in *Eriocheir japonicus* (Niiyama '37),  $2n=138$  *Hemigrapsus penicillatus* (Niiyama This paper),  $2n=128$  in *Hemigrapsus sanguineus* (Niiyama '38),  $2n=118$  in *Pachygrapsus crassipes* (Niiyama This paper) and  $2n=106$  in *Plagusia dentipes* (Niiyama '37a,b). All these species have an XY sex-mechanism in a distance pairing. Considered from the chromosome number and the condition of sex-chromosomes, it seems probable that the Family Grapsidae is a group slightly separated from the other families of the Brachyura. The XO sex-mechanism which was found to occur in a species of the Family Portunidae is a unique example. Decision as to the relationship of this family to the others must be postponed until the observations on many species of this family have completed.

The Reptantia are in general divided into two classes on the basis of chromosome count.

One class is characterized by more or less than 200 chromosome, while the other by 100 or thereabouts. In dimensions the cells are larger in the former class than in the latter. There is, however, an exception in that *Ranina ranina* has large sized cells comparable to those of *Panulirus japonicus*, in spite of the fact that it belongs to the Brachyura with 106 chromosomes in diploid (Niiyama '42).

As is noted in the above discussion, the basic cytological data for understanding the systematic relationship in the Decapoda are very incomplete at present. Under the present status of cytological knowledge, it is very difficult to reach any definite conclusion to generalize the relationship between taxonomical classification and cytological features: a much more complete picture of cytotaxonomy may be expected after an extensive collection of cytological data in many forms of animals.

## 2) Order Isopoda

Reference to Table 1 indicates that, in the Order Isopoda, reports on the chromosomes of twenty-eight species have so far been published.

Vandel ('41), on the basis of his extensive studies, expressed the view that the haploid number of the marine isopods and of the related terrestrial and fresh-water species is 28 or thereabouts. According to him the huge chromosomal elements found in species of *Asellus* are to be regarded as having resulted from a fusion of several small chromosomes as found in *Stenasellus*, though there is no positive proof for that view. Surely, all the chromosomes of *Asellus aquaticus* are large metacentric ones. Vandel states that the chromosome numbers of the true Oniscoidea tend to increase with the evolution of the species: the following relationship seems to occur in the chromosome number of isopods.

Marine species	( $n, 28$ )	.....	<i>Asellus</i> ( $n, 8$ )	.....	Oniscidae	.....	<i>Porcellio</i>	.....	<i>Armadillidium</i> ( $n, 28$ )
			<i>Trichoniscus</i>		( $n, 16$ to $24$ )				

The two extreme forms show a high chromosome number, while the intermediate species have a smaller number, which have probably resulted from fusion or association of several chromosomes.

Looking upon the data presented in Table 1, it becomes evident that among marine species, *Cleantiella isops*,  $n=32$  (Niiyama '59), *Idotea japonicus*,  $n=32$  (Niiyama '59), *Cymodoce japonicus*,  $n=29$  (Niiyama '59) and *Tecticeps japonicus*,  $n=31+Y$  (Niiyama '56), have high chromosome number, while two subspecies of *Jaera marina* with  $n=8+XYY$  and  $n=12+XYY$  (Steiger and Bocquet '54), show a number lower than 28. Certain terrestrial and half-terrestrial species, also, have a high number of chromosome: for instance, *Ligidium hypnorum*,  $n=31$  (Mir '39), *Ligia oceanica*,  $n=30$  (Mir '39), *Tylos granulata*,  $n=34$  (Niiyama '59) and *Megaligia exotica*,  $n=36$  (Niiyama '59) are available for reference. In the authors' opinion, the relationship between the chromosome number and the taxonomical situation in the isopod Crustacea is difficult to establish in the present status of investigation. Sufficient information needful for a decision can not be expected until

after the chromosomes of many species have been exhaustively investigated. The data on the chromosomes of isopod Crustacea are too scanty at present to justify a generalization as to the relationship between the chromosome number and taxonomical arrangement of the species. There is no positive evidence upon which to enter into a consideration of reduction in the chromosome number as a result of chromosome fusion.

### 3) Order Amphipoda

The data shown in Table 1 indicate that cytological investigations have been made on twenty-three species in the Amphipoda by several authors. The studied species are all restricted and belong to two families, Gammaridae and Talitridae of the Suborder Gammaridea. In order to formulate any rule for general application concerning the relationship between cytological data and taxonomical situation, it is necessary to accumulate additional accurate cytological data on many species covering many families of this order.

It is evident from the data in Table 1 that the haploid number of all the species of the Family Talitridae is  $n=25$  in the male (Poisson et Le Calvez '48 : Le Calvez et Certain '51), while in the Family Gammaridae, the fundamental number seems to be  $n=26$ . Almost all the species studied have 26 counts in haploid. Le Calvez ('51) stated that the haploid number, 25, occurring in two species of *Niphargus* is caused by the fusion of chromosomes, since one pair of large V-shaped metacentric chromosomes exists among the spermatogonial garniture (Le Calvez et Certain '51). Le Calvez et Certain ('51) suggested with considerable justification that both Palmer's and Le Roux's determinations, a haploid number of 13 for *Gammarus chevreuxi* (Palmer '25, '26), and *Gammarus duebeni* (Le Roux '33), were based on inadequate technique and were erroneous. There are several species which deviate from the fundamental number ( $n, 26$ ); viz., two species of *Maera* have  $n=32$  (Le Calvez et Certain '51), *Gammarus pungens* has  $n=24$  (Le Calvez et Certain '51) and *Gammarus duebeni* shows  $n=27$  (Orlan and Callan '57, Le Calvez et Certain '51). Le Calvez et Certain ('51) express the opinion that the reduced number is caused by disappearance of some chromosomes by their transformation into heterochromatin.

Orlan and Callan ('57) reported evidence of chromosomal polymorphism to occur in three species of the Gammaridae in the second oocyte showing that there occur  $n, 26$  and  $27$  in *Gammarus pulex*,  $n, 25$  and  $26$  in *Marinogammarus marinus*, and  $n, 29, 30, 31,$  and  $32$  in *Marinogammarus pirloti*. According to their view, there is no evidence to show that this polymorphism is in accord with Robertson's 'fragmentation-fusion' system, but it appears to be due to the occurrence of supernumerary chromosomes. To the present author's view, there is a question whether the number of chromosomes has been correctly counted in the second oocyte division in aceto-orcein squash preparations.

At the present status of investigation, it seems quite difficult to formulate any rule for general application as to the relationship between the chromosome number and taxonomical arrangement of species in these orders, since the basic data for understanding

Table 2. Sex-determining mechanism so far reported in the Crustacea

Species	2n	Sex-chrom.	Author
<b>Phyllopoda</b>			
<i>Branchipus vernalis</i>	23	X-O ♂	Baker & Rosof '27, '28a, b
<b>Ostracoda</b>			
<i>Cyclocypris globosa</i>		X-O ♂	Dietz '58
<i>Cyclocypris laevis</i>		X-Y ♂	Dietz '58
<i>Cyclocypris ovum</i>		X <sup>-3-6</sup> Y or X <sup>-4-7</sup> Y ♂	Dietz '58
<i>Cyprina exsculpta</i>		X <sub>1-3</sub> -Y or X <sub>1-3</sub> X <sub>4</sub> -Y ♂	Dietz '58
<i>Cyprina ophthalmica</i>		X <sub>1-3</sub> X <sub>4</sub> -Y ♂	Dietz '58
<i>Cypris compacta</i>		X <sub>1-3</sub> -Y ♂	Dietz '58
<i>Cypris dietzi</i>		X <sub>1-3</sub> -Y ♂	Dietz '58
<i>Cypris fodiens</i>		X <sub>1-3</sub> -Y ♂	Dietz '58
<i>Cypris whitei</i>		X <sub>1-3</sub> -Y ♂	Dietz '58
<i>Heterocypris incongruens</i>		X <sub>1-6</sub> -Y ♂	Bauer '40
<i>Notodromas monacha</i>		X <sub>1-2</sub> -O ♂	Dietz '54
<i>Physocypris kliei</i>		X <sub>1-3</sub> -O ♂	Dietz '58
<i>Platycypris baueri</i>		X <sub>1-3</sub> -O ♂	Dietz '58
<i>Scottia browniana</i>		X <sub>1-3</sub> -O ♂	Dietz '58
<b>Copepoda</b>			
<i>Centropagis typicus</i>		X-O ♂	Heberer '32
<i>Cyclops affinis</i>		*X-O ♀	Matschek '10
<i>Cyclops fuscus</i> var. <i>distinctus</i>		*X-O ♀	Matschek '10
<i>Cyclops phaleratus</i>	13	*X-O ♀	Braun '09
<i>Cyclops phaleratus</i>		*X-O ♀	Matschek '09, '10
<i>Cyclops prasinus</i>	11	*X-O ♀	Braun '09
<i>Cyclops prasinus</i>		*X-O ♀	Matschek '10
<i>Cyclops serrulatus</i>		*2X-O ♀	Matschek '10
<i>Cyclops vernalis</i>		*X-O ♀	Matschek '09 '10
<i>Ectocyclops strenzki</i>		X-O ♀	Beervann '54
<b>Isopoda</b>			
<i>Asellus aquaticus</i>	17	*X-O ♂	Dworak '35
<i>Jaera marina forsmanni</i>		*X-Y <sub>1</sub> Y <sub>2</sub> ♀	Steiger & Bocquet '54
<i>Jaera marina ischiosetose</i>		*X-Y <sub>1</sub> Y <sub>2</sub> ♀	Steiger & Bocquet '54
<i>Tecticeps japonicus</i>	63	*X-O ♂	Niiyama '56
<b>Amphipoda</b>			
<i>Anisogammarus annandalei</i>	54	X-Y ♂	Niiyama '50
<i>Gammarus chevreuxi</i>	26	*X-Y ♂	Palmer '25, '26
<b>Decapoda</b>			
<i>Cambarus immunis</i> (?)		*8X-O ♂	Fasten '14
<i>Cervimunida princeps</i>	109	X <sub>1</sub> X <sub>2</sub> -Y ♂	Niiyama '59
<i>Eriocheir japonicus</i>	148	X-Y ♂	Niiyama '37
<i>Gaetice depressus</i>	152	X-Y ♂	Niiyama This paper
<i>Hemigrapsus sanguineus</i>	128	X-Y ♂	Niiyama '38
<i>Hemigrapsus penicillatus</i>	138	X-Y ♂	Niiyama This paper
<i>Ovalipes punctatus</i>	103	X-O ♂	Niiyama '40
<i>Pachygrapsus crassipes</i>	118	X-Y ♂	Niiyama This paper
<i>Pandalus borealis</i>	34	*2X-O ♂	Leopoldseder '34
<i>Plagusia dentipes</i>	106	X-Y ♂	Niiyama '37 a, b
<i>Telphusa fluviatilis</i>	78	*2X-O ♂	Delpino '34

\* The data regarding which there is some doubt are marked with asterisks. For reference, see Makino's list, 1956.

the matter are very incomplete. It is necessary to accumulate additional accurate cytological data on a very large scale in the Crustacea in general.

## VI. PART III. SEX-DETERMINING MECHANISM IN THE CRUSTACEA

The vast majority of higher animals have separate sexes and possess genetic mechanism for their sex-determination. Genetic sex-determining mechanisms are of many different kinds. Extensive cytological investigations during more than fifty years since 1900 have presented the fact that there occur two major types in cytological mechanism of sex-determination in animals. The one is referred to as male heterogamety which is characterized by heterogametic sex-chromosomes occurring in the male, while the other deals with female heterogamety which involves a heterogametic condition existing in the female. Accurate data on male heterogamety have been ascertained from both genetical and cytological standpoints to occur in almost all the orders of the Insecta (Arthropoda), while clear cytological and genetical proof for female heterogamety in insects has been offered in two orders, Lepidoptera and Trichoptera. As given in the following, the sex-determining mechanism has been reported cytologically in many species of Crustacea covering six orders, as having either male or female heterogamety. The data are arranged in the form of a table (Table 2).

By reference to Table 2, it becomes evident that, except for certain questionable reports, the sex-determining mechanism in the Crustacea is of XO- or XY-type. In certain species of the Decapoda, Amphipoda and Phyllopoda, the XO- or XY mechanism was found to occur in the male (Baker and Rosof '27, '28a,b, Niiyama '37a, b, '38, '40, '50, '56). Very recently, an  $X_1X_2$ -Y sex-mechanism was reported on the basis of observations of male germ-cells of *Cervimunida princeps* by the present author (Niiyama '59). It has been shown by cytologists (White '40, '41, '54, Cooper '46, '51, Hughes-Schrader '47, '50) that the XX-Y mechanism was secondarily derived from either an originally XO- or an XY condition by translocation involving an autosome and sex-chromosomes. In the Copepoda, male heterogamety of the XO-type was reported by Heberer ('32), while Beervann ('54) observed female heterogamety of the XO-type. In the Ostracoda, besides the XY-type (*Cyclocypris laevis*, Dietz '58) and XO-type (*Cyclocypris globosa*, Dietz '58), a compound sex-chromosomes was reported in males of 12 species, such as  $X_{1-6}$ -Y in *Heterocypris incongruens* (Bauer '40),  $X_{1-2}$ -O in *Notodromas monacha* (Dietz '54),  $X_{1-3}$ -Y in four species of *Cypris*, *Physocypris kliei*, *Platycypris baueri* and *Scottia browniana*  $X_{3-6}$ -Y or  $X_{4-7}$ -Y in *Cyclocypris ovum* and  $X_{1-3}$ -Y or  $X_{1-3}X_4$ -Y in *Cypris exsculpta* and  $X_{1-3}X_4$ -Y in *Cypris ophthalmica* (Dietz '58). Similar situation was found to occur also in some species of insects (see Makino's list 1956).

The remarkably cytological feature is the evidence of distance pairing of the XY sex-chromosomes mechanism which was found to exist throughout all the species of the Family Grapsidae so far studied. The distance pairing was also found to occur in the

$X_1X_2$  and Y sex-chromosomes of *Cervimunida princeps* belonging to the Family Galateidae, as described in the foregoing pages. In none of the cases above noted was the establishment of distance pairing was observed with certainty in the meiotic prophase, since there appear at diakinetiic prophase many autosomal chromatic bodies which are indistinguishable in size from  $X_1$ ,  $X_2$  and Y in the nucleus. In *Anisogammarus annandalei* the association of the X and Y by means of connecting fibres was found in the diakinetiic stage. The connecting fibres seem to contract by and by with the advance in stage in the meiotic prophase nucleus and as a result, the two elements come to lie in the equatorial plate assuming an end-to-end conjugation with their long axes at right angle to the plate at metaphase.

Based on the evidence presented above the author considers that the distance pairing of the sex-chromosome at metaphase depends on the degree of contraction of the connecting fibres between the two sex-elements. This is suggested by the fact that the distance between the X and Y pair at metaphase is dissimilar in different species of Family Grapsidae. The distance between the X and Y pair is greatest in *Hemigrapsus sanguineus* (Figs. 105-106) and *Gaetice depressus* (Figs. 127-128), while in *Hemigrapsus penicillatus* (Figs. 112-114) and *Plagusia dentipes* (Figs. 137-140) it ranks next. *Eriocheir japonicus* (Figs. 120-121) and *Pachygrapsus crassipes* (Figs. 97-99) show the smallest distance between them. To the extreme case, the X and Y show no evidence of distance pairing, an example of which was found in *Anisogammarus annandalei* (Fig. 187). Though the X and Y show no evidence of distance pairing in the latter form, the sex-elements are clearly distinguishable from the autosomal elements by the fact that the two differ considerably in size. If the size-difference between the X and Y is very small or nearly identical in magnitude, the X and Y could not be identified among the autosomes in the complex. The case may be found, to the author's view, in the majority of species of the Crustacea in which there is no evidence for the existence of the sex-chromosomes. Generally speaking it is quite impossible to find out in the Crustacea the sex-chromosome by the usual method of alignment arrangement of homologous chromosomes in a complex, since there are many chromosomes diminishing in size gradually from rod to minute spherule in a complex, and accordingly there is a difficulty in identifying the homologous mates.

*Ovalipes punctatus* is characterized by the XO-type sex-mechanism. It is remarkable that the X chromosome of the present species always lie in the central position of the equatorial plate, considerably apart from the equatorial plate. The position of the X of this species is nearly identical with that of the X found in several species of the Grapsidae which show the XY-mechanism. The impression is given that the unusual position of the X in *Ovalipes punctatus* is of the ancestral type deviated from the distance pairing of the XY mechanism. It seems very probable to the author that the ancestor of *Ovalipes punctatus* may have had XY-mechanism showing the distance pairing as occurs in many species of the Grapsidae, and that the disappearance of the Y may have occurred in

comparatively later time in the course of evolution, or that Y still remains in the state of a heterochromatin. Based on the above considerations, it is likely that the XY sex-chromosomes occurring in the Grapsidae and the XO sex-chromosome existing in the *Ovalipes punctatus* may have had a similar origin in the evolutionary development.

In contrast to the XO sex-chromosomes as discussed above, the sex-mechanism found in *Tecticeps japonicus* is of a type commonly occurring among insects (see Makino's list, 1956). It seems apparent that such an XO-mechanism may have resulted from the disappearance of the Y chromosome long ago. The fact that the X chromosome lies far apart from the equatorial plate at metaphase may not be due to the precession of that chromosome in division.

Rather frequently a clear constriction is found to occur in the middle portion of the X chromosome of *Plagusia dentipes* and *Eriocheir japonicus*: this finding seems to suggest a close relation between the XY-mechanism and XX-Y mechanism of sex-chromosomes since such a constriction of the X-chromosome may be due to the existence of a compound nature of the chromosome. If the two components of a compound X are separated there may occur two X's called  $X_1$  and  $X_2$ . This is strongly supported by the assumption of White ('40, '41, '54), Cooper ('46, '51), and Hughes-Schrader ('47, '50) who believed that the  $X_1X_2Y$ -mechanism was secondarily derived from either an originally XO- or XY-condition by translocation involving an autosome and sex-chromosomes. The X having a constriction as occurred in *Plagusia dentipes* (Figs. 137-140) and *Eriocheir japonicus* (Figs. 120-121), is thick in appearance, while the X-element of XY-type occurring in *Hemigrapsus sanguineus* (Figs. 105-106) is slender in form. The former X chromosome of the former type may be produced as a result of translocation involving an autosome and sex-chromosomes of the latter type.

The evolutionary process of the sex-chromosome, therefore, seems to proceed two ways from the XY-type occurring in *Hemigrapsus sanguineus*. As a result of the disappearance of the Y in a certain mechanism from the XY-mechanism found in *Hemigrapsus sanguineus*, there may be produced the XO-type which occurs in *Ovalipes punctatus*, is one way. And in another way, by the association of a pair of autosomes with the sex-chromosome as occurs in *Hemigrapsus sanguineus*, the XY-mechanism occurring in *Plagusia dentipes* and *Eriocheir japonicus* may result. If the X chromosome of *Plagusia dentipes* is divided into two components, there may be derived an  $X_1X_2Y$ -type as found in *Cervimunida princeps*. If the XY chromosomes having a compound nature cast off the Y-element during the evolutionary process, the XO-type mechanism as found in *Tecticeps japonicus* may be produced. The association of the sex-chromosome with a pair of autosomes might occur on the one hand and on the other hand the disappearance of the Y chromosome might occur repeatedly, so the multiple X occurring in the Ostracoda will appear (Bauer '40, Dietz '54, '58).

In conclusion, though the elucidation of the evolutionary process of the sex-

Table 3. The species under study and their chromosome numbers

Decapoda	2n	Formula	n
<i>Penaeus japonicus</i>	92	14V'+78R'	46
<i>Panulirus japonicus</i>	140	12V'+128R'	70
<i>Cambarus clarkii</i>	192	6V'+186R'	96
<i>Cambaroides japonicus</i>	196	196 rods	98
<i>Nephrops japonicus</i>	164	164 rods	82
<i>Nephropsis carpenteri</i>	152	30V'+122R'	76
<i>Cervimunida princeps</i>	109	18V'+91R'	53+X <sub>1</sub> X <sub>2</sub> Y (♂I)
<i>Eupagurus ochotensis</i>	254	18V'+236R'	127
<i>Coenobita rugosa</i>	230	230 rods	
<i>Paralithodes camtschatica</i>	208	208 rods	104
<i>Paralithodes platypus</i>	206	18V'+188R'	103
<i>Matuta lunaris</i>	94	4V'+90R'	47
<i>Philyra pisum</i>	114	4V'+110R'	57
<i>Ranina ranina</i>	106	24V'+82R'	53
<i>Macrocheira kaempferi</i>	106	16V'+90R'	53
<i>Ovalipes punctatus</i>	103	4V'+99R'	51+XO (♂I)
<i>Scylla serrata</i>	106	106 rods	53
<i>Telmessus cheiragonus</i>	124	18V'+116R'	62
<i>Atergatis floridus</i>	104	4V'+100R'	52
<i>Pachygrapsus crassipes</i>	118	118 rods	58+XY (♂I)
<i>Hemigrapsus sanguineus</i>	128	128 rods	63+XY (♂I)
<i>Hemigrapsus penicillatus</i>	138	2V'+136R'	68+XY (♂I)
<i>Eriocheir japonicus</i>	148	148 rods	73+XY (♂I)
<i>Gaetice depressus</i>	152	12V'+140R'	75+XY (♂I)
<i>Sesarma intermedia</i>	102	102 rods	
<i>Plagusia dentipes</i>	106	106 rods	52+XY (♂I)
Isopoda			
<i>Megaligia exotica</i>	72	12V'+60R'	36
<i>Tylos granulata</i>	68	20V'+48R'	34
<i>Cleantiella isops</i>	64	34V'+30R'	32
<i>Idotea japonica</i>	64	30V'+34R'	32
<i>Cymodoce japonica</i>	58	32V'+26R'	29
<i>Tecticeps japonicus</i>	63	27V'+36R'	31+XO (♂I)
Amphipoda			
<i>Anisogammarus annandalei</i>	54	54 rods	26+XY (♂I)

mechanism remains incomplete at present due to the poor data, the impression is given that the sex-chromosomes of the Crustacea exhibit an evolutionary development showing a connection with the taxonomical relationship.

### SUMMARY

The present study is divided into three parts. Part I is devoted to the description of the comparative morphology of chromosomes in 33 species of the Decapoda, Isopoda and Amphipoda of Crustacea. The species under study and the number of chromosomes established by the present author are compiled in Table 3.

Part II deals with the chromosome morphology in relation to taxonomical classification of the Crustacea, by reference to the cytological data so far presented by cytologists. The numerical relationship of the chromosomes so far established in the Crustacea is shown

as a summary in Table 1.

The Decapoda, though there occurs a wide range of variation in the number of chromosomes, seems to be differentiable into two classes on the basis of chromosome counts. One class of decapods is characterized by more or less 200 chromosomes, while the other by 100 or thereabout.

In the Isopoda the chromosome number varies rather widely ranging from  $2n$ , 12 to  $2n$ , 72. Vandell states that the chromosome number of the true Oniscoidea shows a tendency to increase with the evolution of species, and that two extreme forms show a high chromosome number, while the intermediate species have a smaller number. There are, however, considerable exceptions which show variation from Vandell's rule. In the author's opinion, it is difficult to find any rule for a generalization of the relationship between taxonomical classification and cytological features in the present order.

In the Amphipoda, on the other hand, the counts show comparatively little variation. Though there are some exceptions, it was decided that the fundamental number of chromosome of the Family Gammaridae is 26 ( $n$ ) and that of the Family Talitridae is 25 ( $n$ ).

In Part III is discussed the sex-determining mechanism in the Crustacea. It has been reported on the basis of the cytological study that the majority of crustacean species show male heterogamety, while in certain forms female heterogamety occurs. The data so far accumulated are shown in Table 2. Disregarding certain reports open to doubt, one may say that the sex-determining mechanism of the Crustacea is of the XO-, XY- type or modifications thereof. In several species of the Decapoda, Amphipoda and Phyllopoda, the XO-XY and XXY-mechanism was found to occur in the male. In the Copepoda, male heterogamety of an XO-type and female heterogamety of the same type have been reported. In the Ostracoda, a compound sex-chromosome was found to occur in males of 12 species.

The relationship between the X-O, X-Y and XX-Y mechanism was considered on the basis of evolutionary development of the sex-mechanism in the Crustacea in general, and some possible features were discussed of the development of the sex-chromosomes in relation to taxonomy.

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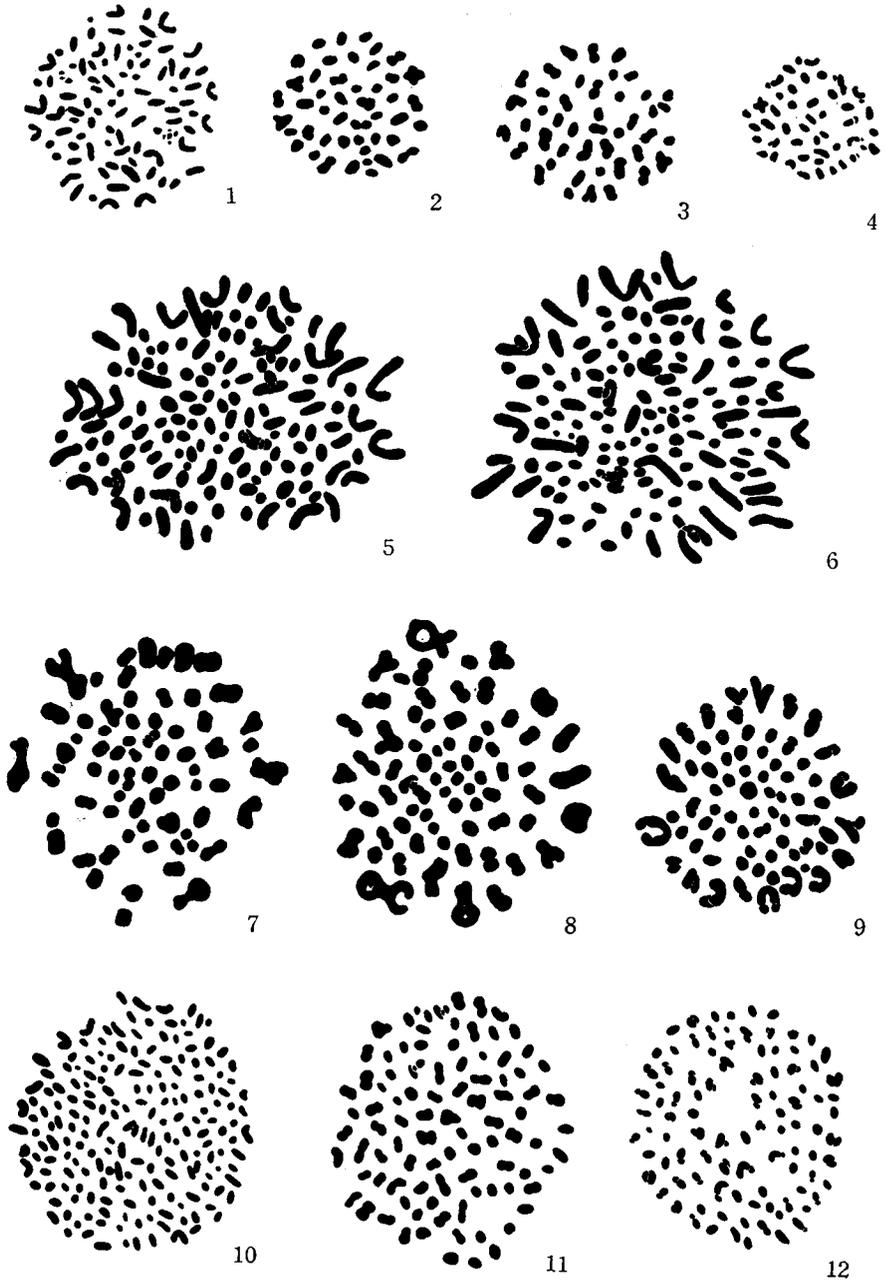
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## EXPLANATION OF PLATES

PLATE I

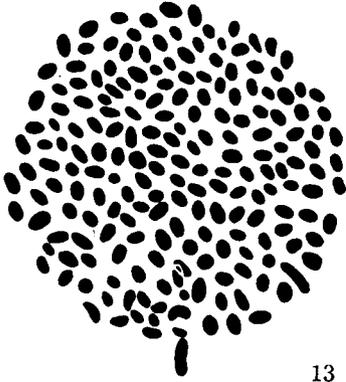
- Figs. 1- 4. Chromosomes of *Panaeus japonicus*.
1. Polar view of spermatogonial metaphase, 92 (14V' + 78R') chromosomes.
  - 2- 3. Metaphase polar views of primary spermatocytes, 46 bivalents in each.
  4. Metaphase polar view of secondary spermatocyt, 46 dyads.
- Figs. 5- 9. Chromosomes of *Panulirus japonicus*.
- 5- 6. Polar views of spermatogonial metaphases, 140 (12V' + 128R') chromosomes in each.
  - 7- 8. Metaphase polar views of primary spermatocytes, 70 bivalents in each. Note three compound ring-tetrads and transvers suturs at middle portions of large bivalents.
  9. Metaphase polar view of secondary spermatocyte, 70 dyads.
- Figs. 10-12. Chromosomes of *Cambarus clarkii*.
10. Polar view of spermatogonial metaphase, 192 (6V' + 186R') chromosomes.
  11. Metaphase polar view of primary spermatocyte, 96 bivalents.
  12. Metaphase polar view of secondary spermatocyte, 96 dyads.



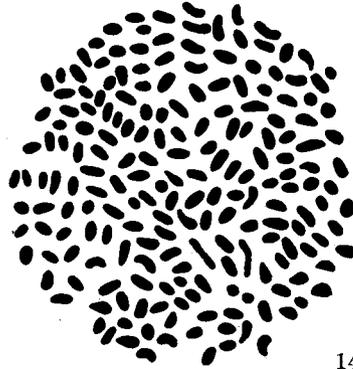
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PLATE II

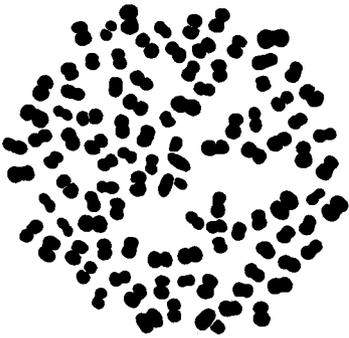
- Figs. 13-18. Chromosomes of *Cambaroides japonicus*.
- 13-14. Spermatogonial complexes, 196 rod-shaped chromosomes in each.
  - 15-16. Metaphase polar views of primary spermatocytes, 98 bivalents in each. Note transverse sutures at middle portions of bivalents.
  - 17. Metaphase polar view of secondary spermatocytes, 98 dyads.
  - 18. One of two daughter chromosome garnitures at anaphase of the secondary divisions, containing 98 monads.



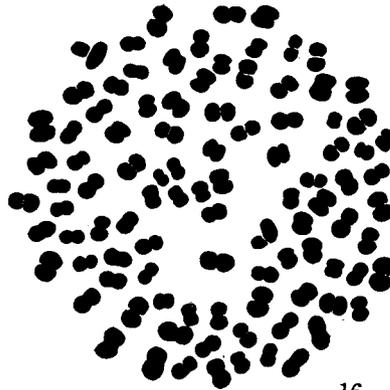
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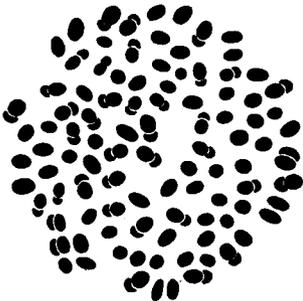
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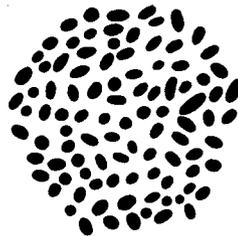
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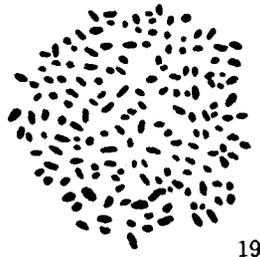
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### PLATE III

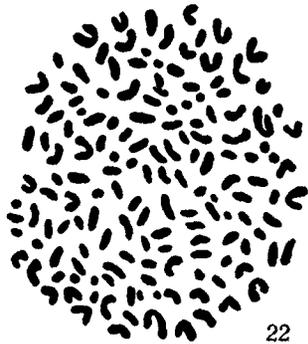
- Figs. 19–21. Chromosomes of *Nephrops japonicus*.
19. Polar view of spermatogonial metaphase, 164 (all rod) chromosomes.
  20. Metaphase polar view of primary spermatocyte, 82 bivalents. Note quadripartite structure of bivalents.
  21. Side-view of primary division at early anaphase.
- Figs. 22–25. Chromosomes of *Nephropsis carpenteri*.
22. Polar view of spermatogonial metaphase, 152 (30V' + 122R') chromosomes.
  - 23–24. Metaphase polar views of primary spermatocytes, 76 bivalents in each. Note quadripartite and hexapartite structure of bivalents.
  25. Side-view of primary division at early anaphase, Note quadripartite, hexapartite and octopartite structure of bivalents.



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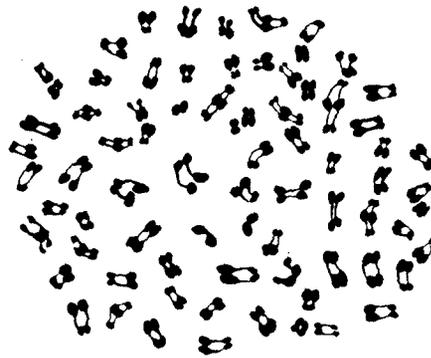
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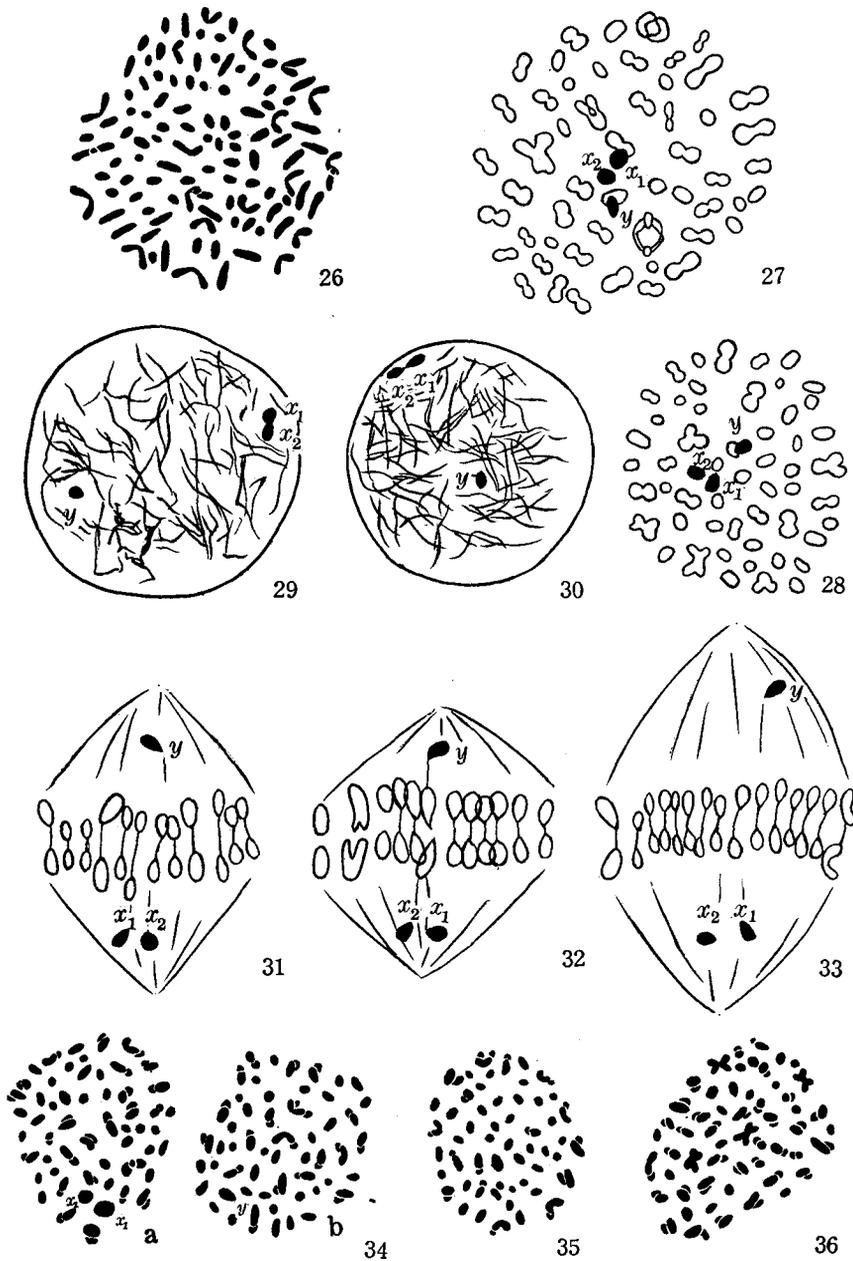
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## PLATE IV

- Figs. 26-36. Chromosomes of *Cervimunida princeps*.
- 26. Metaphase plate of spermatogonium, 109 (18V' + 91R') chromosomes.
  - 27-28. Polar views of metaphase chromosomes of primary spermatocytes, 53 bivalents and X<sub>1</sub>X<sub>2</sub>Y chromosomes in each.
  - 29-30. Nuclei in leptotene stage, showing heteropycnotic bodies consisting of X<sub>1</sub>X<sub>2</sub> and Y in deep black.
  - 31-33. Side views of metaphase chromosomes of primary spermatocytes, indicating distance pairing of X<sub>1</sub>X<sub>2</sub>-Y in each.
  - 34 a-b. Two daughter complexes of the equatorial plate of a primary spermatocyte at anaphase.
  - 35. Metaphase of a secondary spermatocyte, containing 54 chromosomes.
  - 36. The same, with 55 chromosomes.



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PLATE V

- Figs. 37-40. Chromosomes of *Eupagurus ochotensis*.
- 37. Polar view of spermatogonial metaphase, 254 (18V' + 236R') chromosomes.
  - 38-39. Metaphase polar views of primary spermatocytes, 127 bivalents in each.
  - 40. Metaphase polar view of secondary spermatocyte, 127 dyads.
- Fig. 41. Spermatogonial chromosomes at metaphase of *Coenobita rugosa*, 230 rod-shaped chromosomes.
- Figs. 42-43. Spermatogonial garnitures of *Paralithodes camtschatica* at metaphase, 208 chromosomes of rod shaped in each.

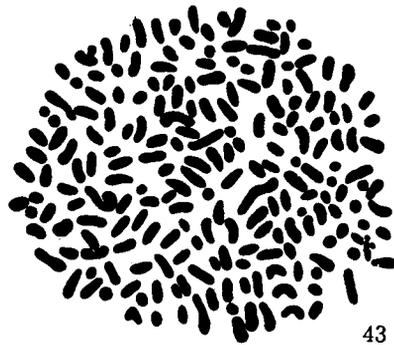
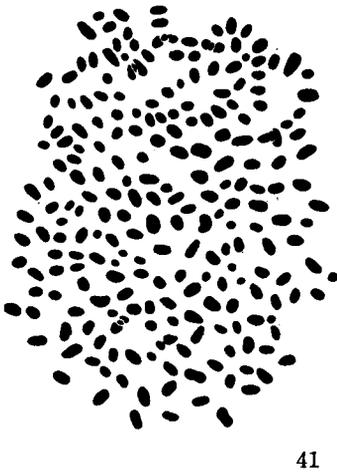
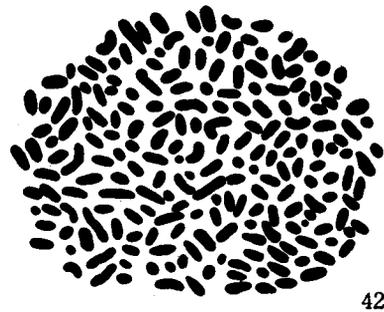
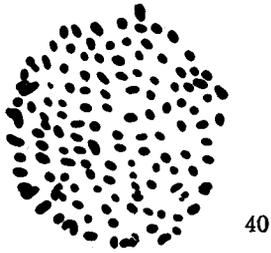
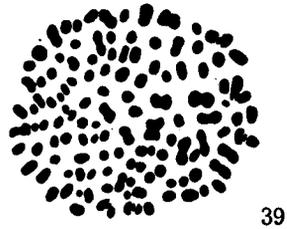
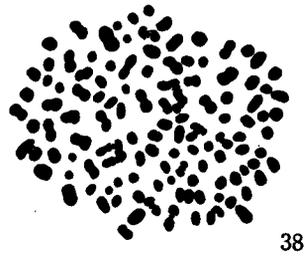
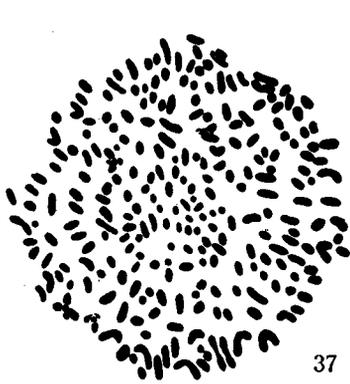
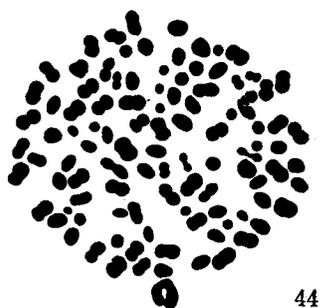
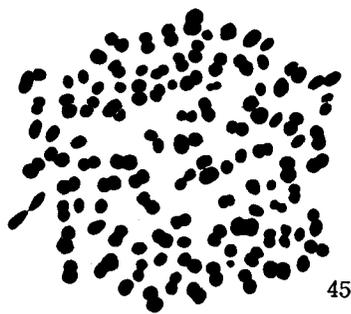


PLATE VI

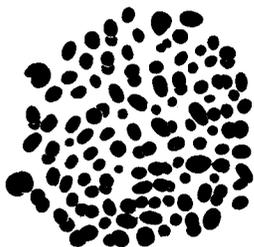
- Figs. 44-47. Chromosomes of *Paralithodes camtschatica*.
- 44-45. Metaphase polar views of primary spermatocytes, 104 bivalents having transverse sutures at their middle portions.
  - 46-47. Metaphase polar views of secondary spermatocytes, 104 dyads in each.
- Figs. 48-51. Chromosomes of *Paralithodes platypus*.
- 48. Polar view of spermatogonial metaphase, 206 (18V' + 188R') chromosomes.
  - 49-50. Metaphase polar views of primary spermatocytes, 103 bivalents in each.
  - 51. Metaphase polar view of secondary spermatocyte, 103 dyads.



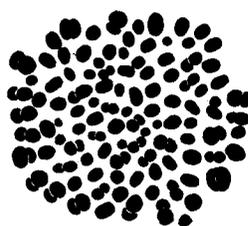
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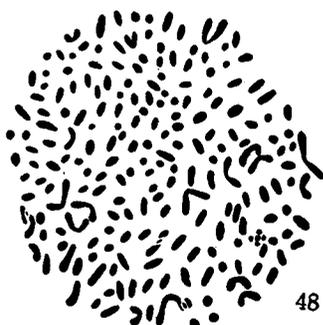
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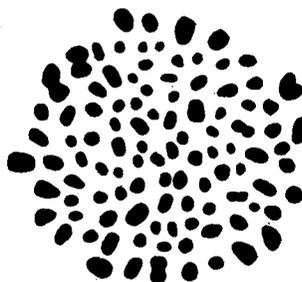
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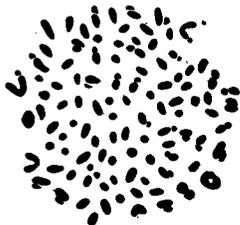
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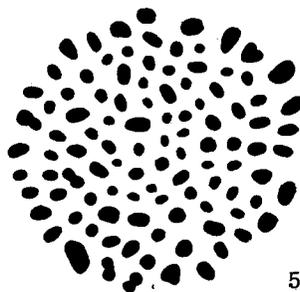
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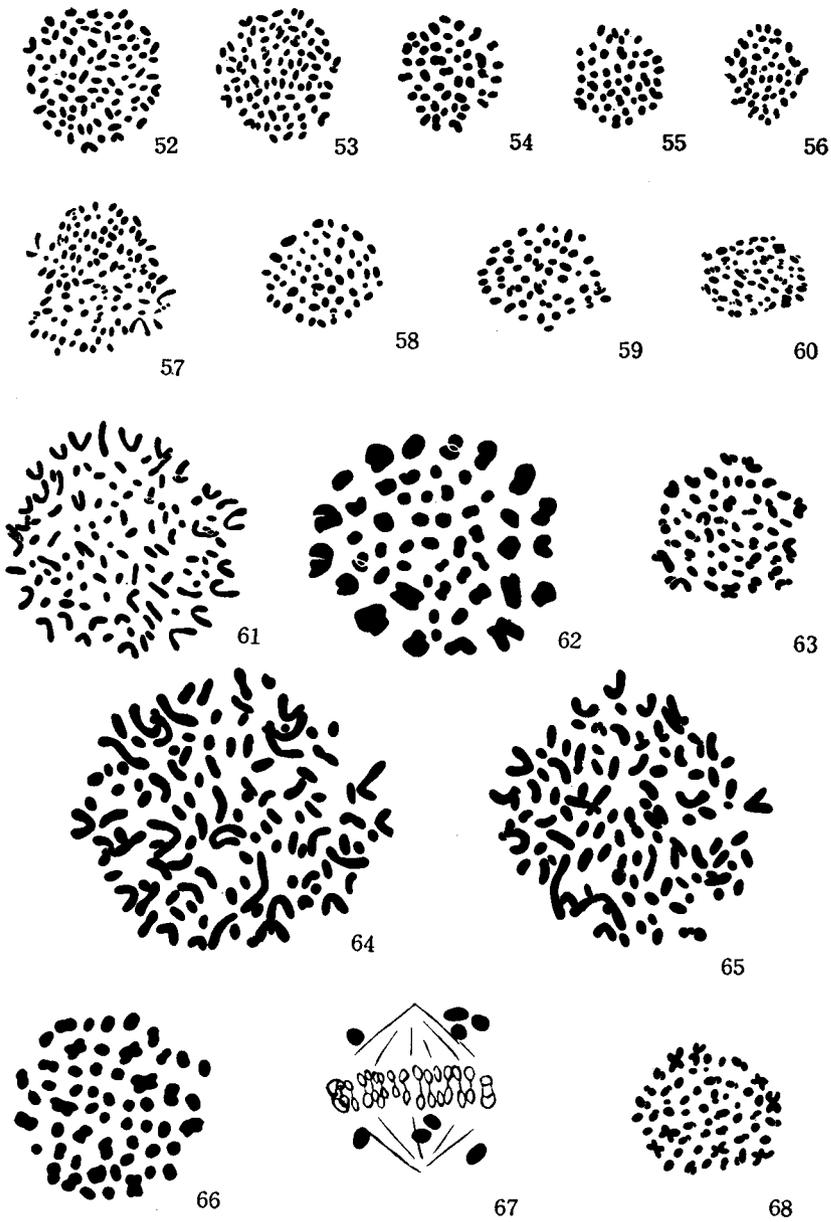
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## PLATE VII

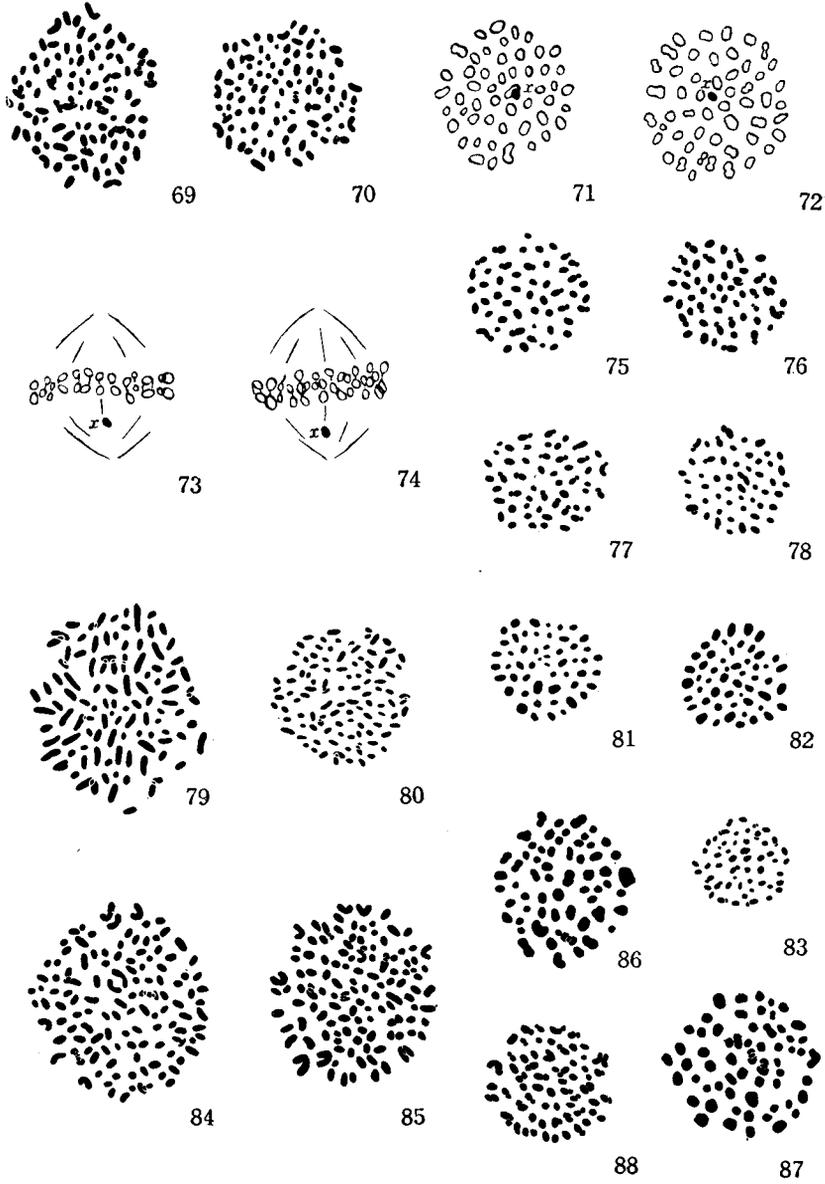
- Figs. 52-56. Chromosomes of *Matuta lunaris*.  
52-53. Polar views of spermatogonial metaphases, 94 (4V'+90R') chromosomes.  
54-55. Metaphase polar views of primary spermatocytes, 47 bivalents in each.  
56. Metaphase polar view of secondary spermatocyte, 47 dyads.
- Figs. 57-60. Chromosomes of *Philyra pisum*.  
57. Polar view of spermatogonial metaphase, 114 (4V'+110R') chromosomes.  
58-59. Metaphase polar views of primary spermatocytes, 57 bivalents in each.  
60. Metaphase polar view of secondary spermatocyte, 57 dyads.
- Figs. 61-63. Chromosomes of *Ranina ranina*.  
61. Polar view of spermatogonial metaphase, 106 (24V'+82R') chromosomes.  
62. Metaphase polar view of primary spermatocyte, 53 bivalents.  
63. Metaphase polar view of secondary spermatocyte.
- Figs. 64-68. Chromosomes of *Macrocheira kaempferi*.  
64-65. Polar views of spermatogonial metaphases, 106 (16V'+90R') chromosomes.  
66. Metaphase polar view of primary spermatocyte, 53 bivalents.  
67. Metaphase side view of primary spermatocyte, indicating chromatoid bodies in deep black.  
68. Metaphase polar view of secondary spermatocyte, 53 dyads.



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PLATE VIII

- Figs. 69-78. Chromosomes of *Ovalipes punctatus*.
- 69-70. Polar views of spermatogonial metaphases, 103 (4V' + 99R') chromosomes in each.
  - 71-72. Metaphase polar views of primary spermatocytes, 51 bivalents and an X chromosome in each.
  - 73-74. Side views of metaphase chromosomes of primary spermatocytes, indicating an X chromosome in precession in each.
  - 75-76. Metaphase polar views of secondary spermatocyte, containing 51 chromosomes in each.
  - 77-78. The same, with 52 chromosomes.
- Figs. 79-83. Chromosomes of *Scylla serrata*.
- 79-80. Polar views of spermatogonial metaphases, 106 rod-shaped chromosomes in each. 79: early spermatogonium. 80: late spermatogonium.
  - 81-82. Metaphase polar views of primary spermatocytes, 53 bivalents in each.
  - 83. Metaphase polar views of secondary spermatocyte, 53 dyads.
- Figs. 84-88. Chromosomes of *Telmessus cheiragonous*.
- 84-85. Polar views of spermatogonial metaphases, 124 (8V' + 116R') chromosomes in each.
  - 86-87. Metaphase polar views of primary spermatocytes, 62 bivalents in each.
  - 88. Metaphase polar view of secondary spermatocyte, 62 dyads.



## PLATE IX

Figs. 89-92. Chromosomes of *Atergatis floridus*.

89. Polar view of spermatogonial metaphase, 104 (4V' + 100R') chromosomes.

90-91. Metaphase polar views of primary spermatocytes, 52 bivalents in each.

92. Metaphase polar view of secondary spermatocyte, 52 dyads.

Figs. 93-100. Chromosomes of *Pachygrapsus crassipes*.

93. Polar view of spermatogonial metaphase, 118 (all rod) chromosomes.

94-96. Metaphase polar views of primary spermatocytes, 58 bivalents and XY chromosomes in each.

97-99. Side views of metaphase chromosomes of primary spermatocyte, indicating distance pairing of X-Y in each.

100. Metaphase polar view of a secondary spermatocyte, 59 dyads.

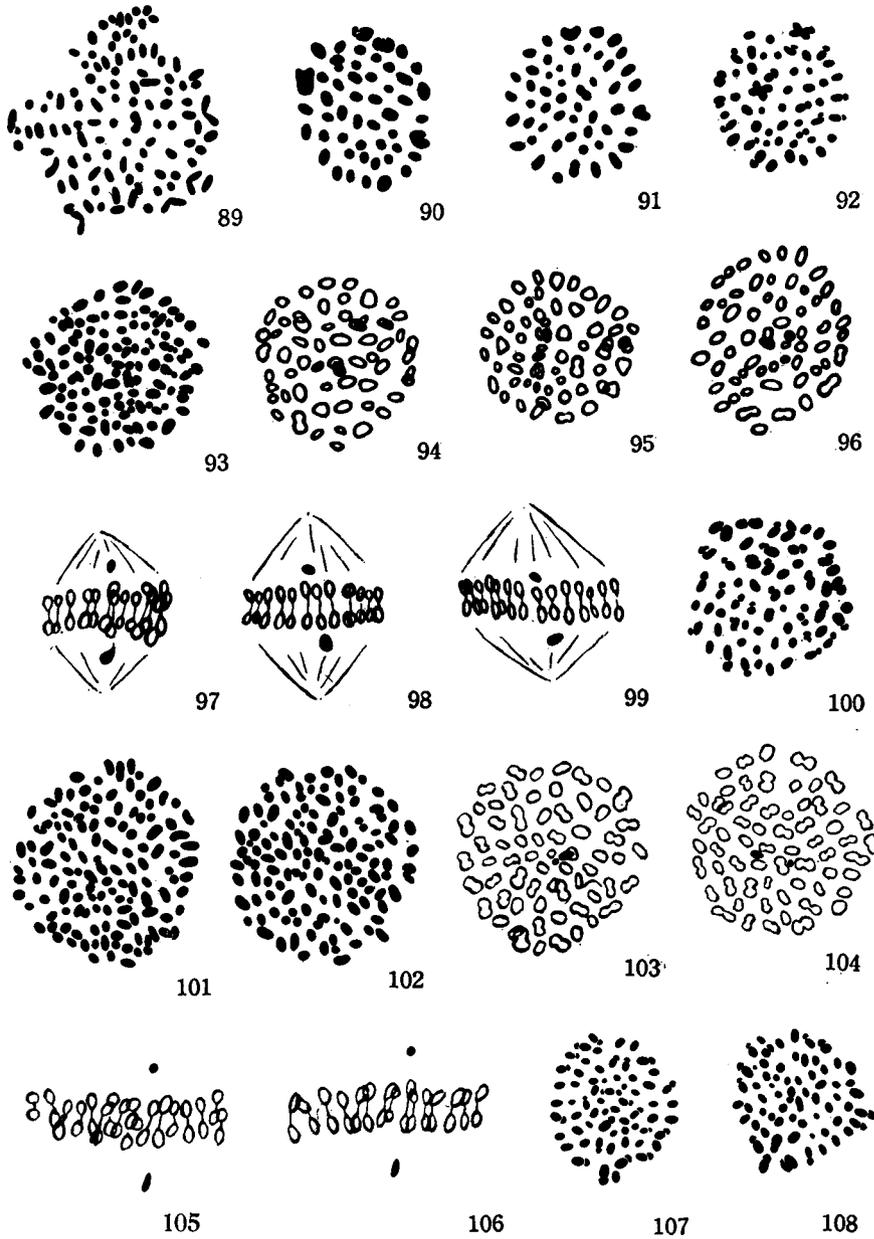
Figs. 101-108. Chromosomes of *Hemigrapsus sanguineus*.

101-102. Polar views of spermatogonial metaphases, 128 (all rod) chromosomes in each.

103-104. Metaphase polar views of primary spermatocytes, 63 bivalents and XY chromosomes in each.

105-106. Side views of metaphase chromosomes of primary spermatocytes, indicating distance pairing of X-Y in each.

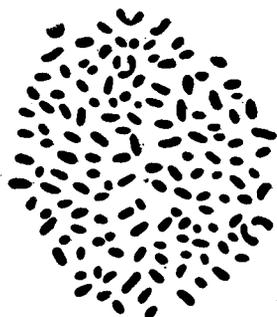
107-108. Metaphase polar views of secondary spermatocytes, 64 dyads in each.



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PLATE X

- Figs. 109–115. Chromosomes of *Hemigrapsus penicillatus*.
- 109. Polar view of spermatogonial metaphase, 138 (2V' + 136R') chromosomes.
  - 110–111. Metaphase polar views of primary spermatocytes, 68 bivalents and XY chromosomes in each.
  - 112–114. Side views of metaphase chromosomes of primary spermatocytes, indicating distance pairing of X-Y in each.
  - 115. Metaphase polar view of secondary spermatocyte, 69 dyads.
- Figs. 116–122. Chromosomes of *Eriocheir japonicus*.
- 116–117. Polar views of spermatogonial metaphases, 148 (all rod) chromosomes in each 116: early spermatogonium. 117: late spermatogonium.
  - 118–119. Metaphase polar views of primary spermatocytes, 73 bivalents and XY chromosomes in each.
  - 120–121. Side views of metaphase chromosomes of primary spermatocytes, indicating distance pairing of X-Y in each.
  - 122. Metaphase polar views of secondary spermatocyte, 74 dyads.
- Fig. 123. Metaphase polar view of spermatogonial chromosomes of *Sesarma intermedia*, containing 102 rod-shaped chromosomes.



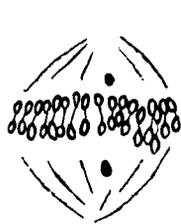
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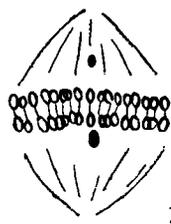
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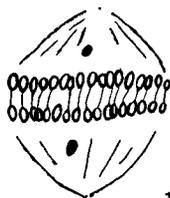
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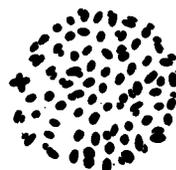
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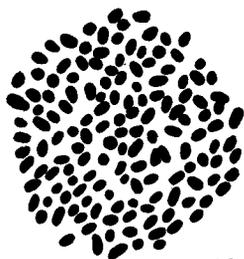
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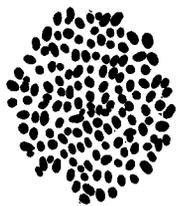
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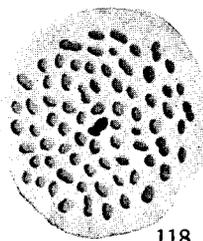
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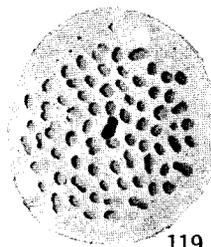
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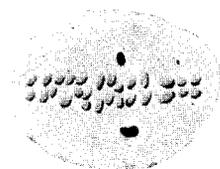
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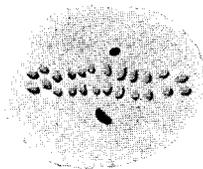
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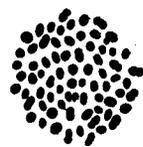
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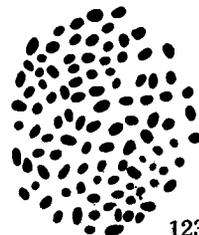
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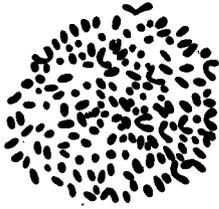
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## PLATE XI

- Figs. 124–129. Chromosomes of *Gaetice depressus*.
- 124. Polar view of spermatogonial metaphase, 152 (12V'+140R') chromosomes.
  - 125–126. Metaphase polar views of primary spermatocytes, 75 bivalents and XY complex in each.
  - 127–128. Side views of metaphase chromosomes of primary spermatocytes, indicating distance pairing X-Y in each.
  - 129. Metaphase polar view of secondary spermatocyte, 76 dyads.
- Figs. 130–144. Chromosomes of *Plagusia dentipes*.
- 130–132. Polar views of spermatogonial metaphases, 106 (all rod) chromosomes in each.
  - 133–136. Metaphase polar views of primary sperm .ocytes, 52 bivalents and XY complex in each.
  - 137–140. Side views of metaphase chromosomes of primary spermatocytes, indicating distance pairing of X-Y in each.
  - 141–144. Metaphase polar views of secondary spermatocytes, 53 dyads in each.



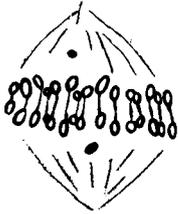
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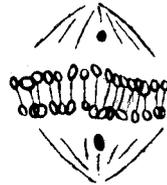
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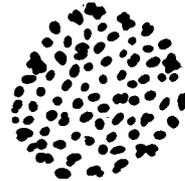
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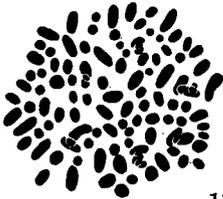
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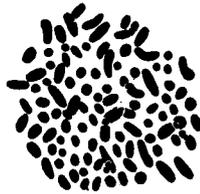
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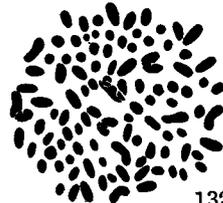
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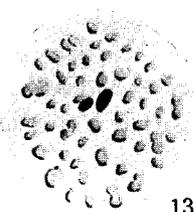
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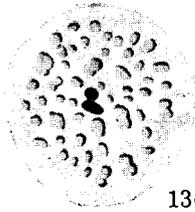
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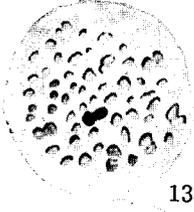
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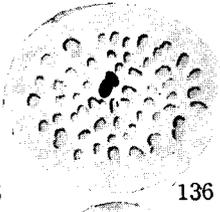
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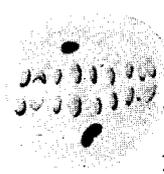
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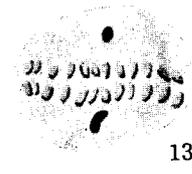
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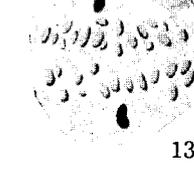
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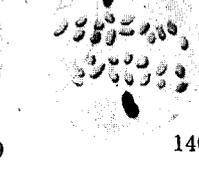
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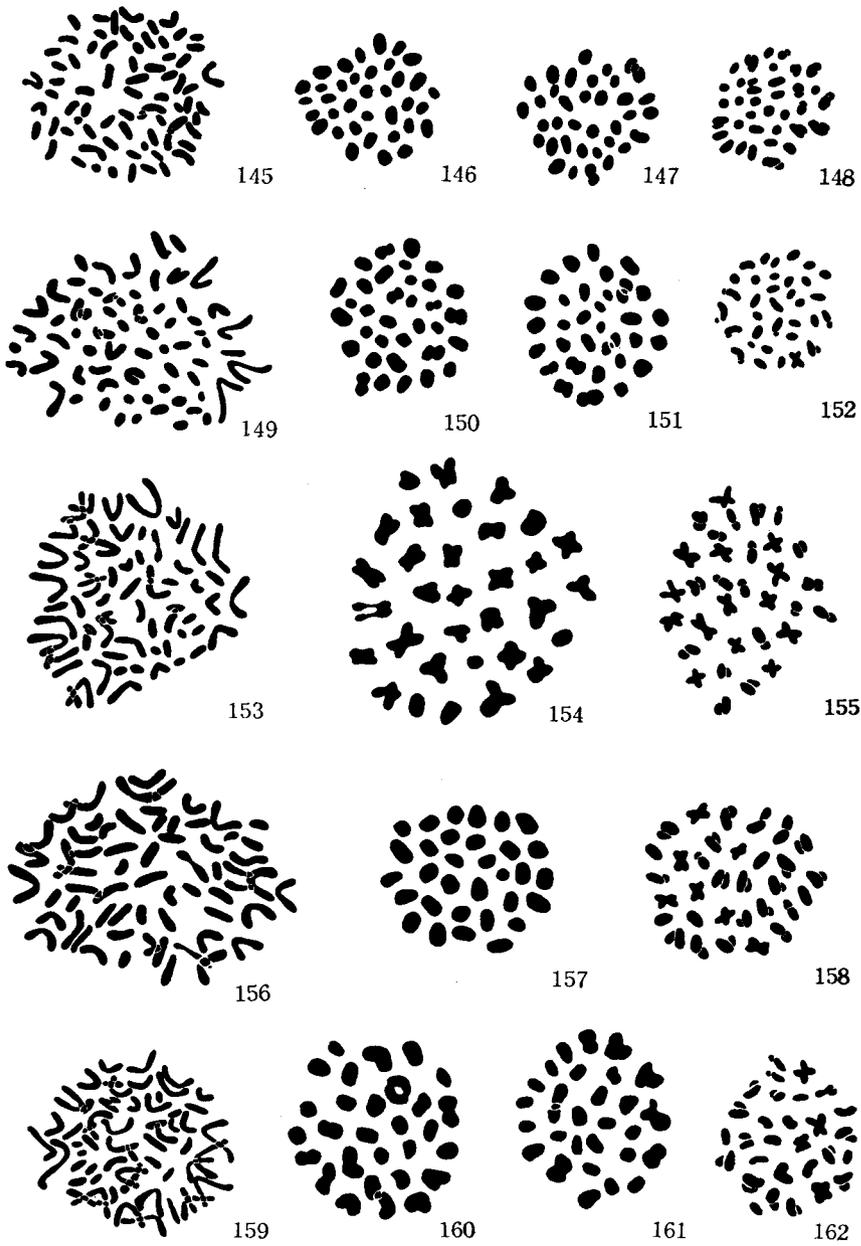
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## PLATE XII

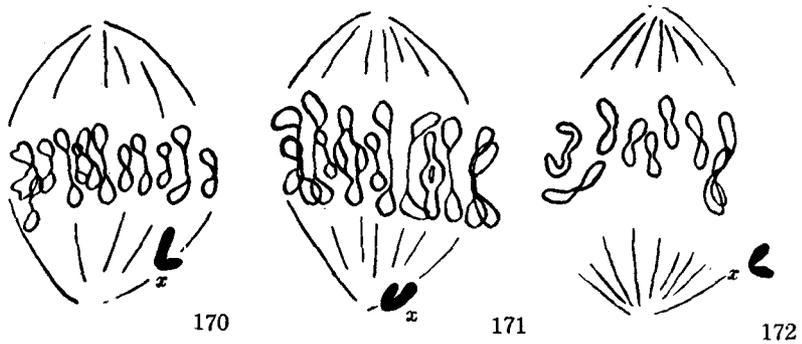
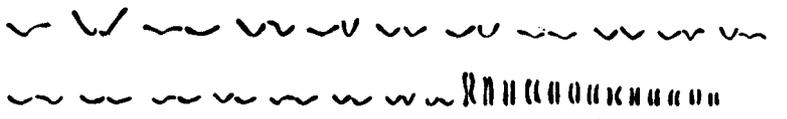
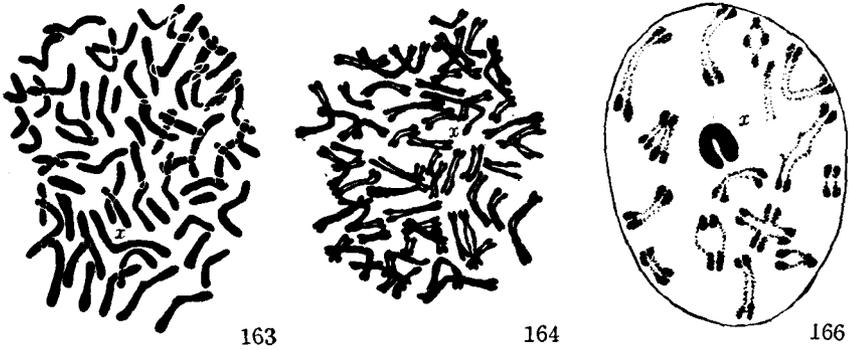
- Figs. 145–148. Chromosomes of *Megaligia exotica*.
- 145. Polar view of spermatogonial metaphase, 72 (12V'+60R') chromosomes.
  - 146–147. Metaphase polar views of primary spermatocytes, 36 bivalents in each.
  - 148. Metaphase polar view of secondary spermatocyte, 36 dyads.
- Figs. 149–152. Chromosomes of *Tylos granulata*.
- 149. Polar view of spermatogonial metaphase, 68 (20V'+48R') chromosomes.
  - 150–151. Metaphase polar views of primary spermatocytes, 34 bivalents in each.
  - 152. Metaphase polar view of secondary spermatocyte, 34 dyads.
- Figs. 153–155. Chromosomes of *Cleantiella isops*.
- 153. Polar view of spermatogonial metaphase, 64 (34V'+30R') chromosomes.
  - 154. Metaphase polar view of primary spermatocyte, 32 bivalents.
  - 155. Metaphase polar view of secondary spermatocyte, 32 dyads.
- Figs. 156–158. Chromosomes of *Idotea japonica*.
- 156. Polar view of spermatogonial metaphase, 64 (30V'+34R') chromosomes.
  - 157. Metaphase polar view of primary spermatocyte, 32 bivalents.
  - 158. Metaphase polar view of secondary spermatocyte, 32 dyads.
- Figs. 159–162. Chromosomes of *Cymodoce japonicus*.
- 159. Polar view of spermatogonial metaphase, 58 (32V'+26R') chromosomes.
  - 160–161. Metaphase polar views of primary spermatocytes, 29 bivalents in each.
  - 162. Metaphase polar view of secondary spermatocyte, 29 dyads.



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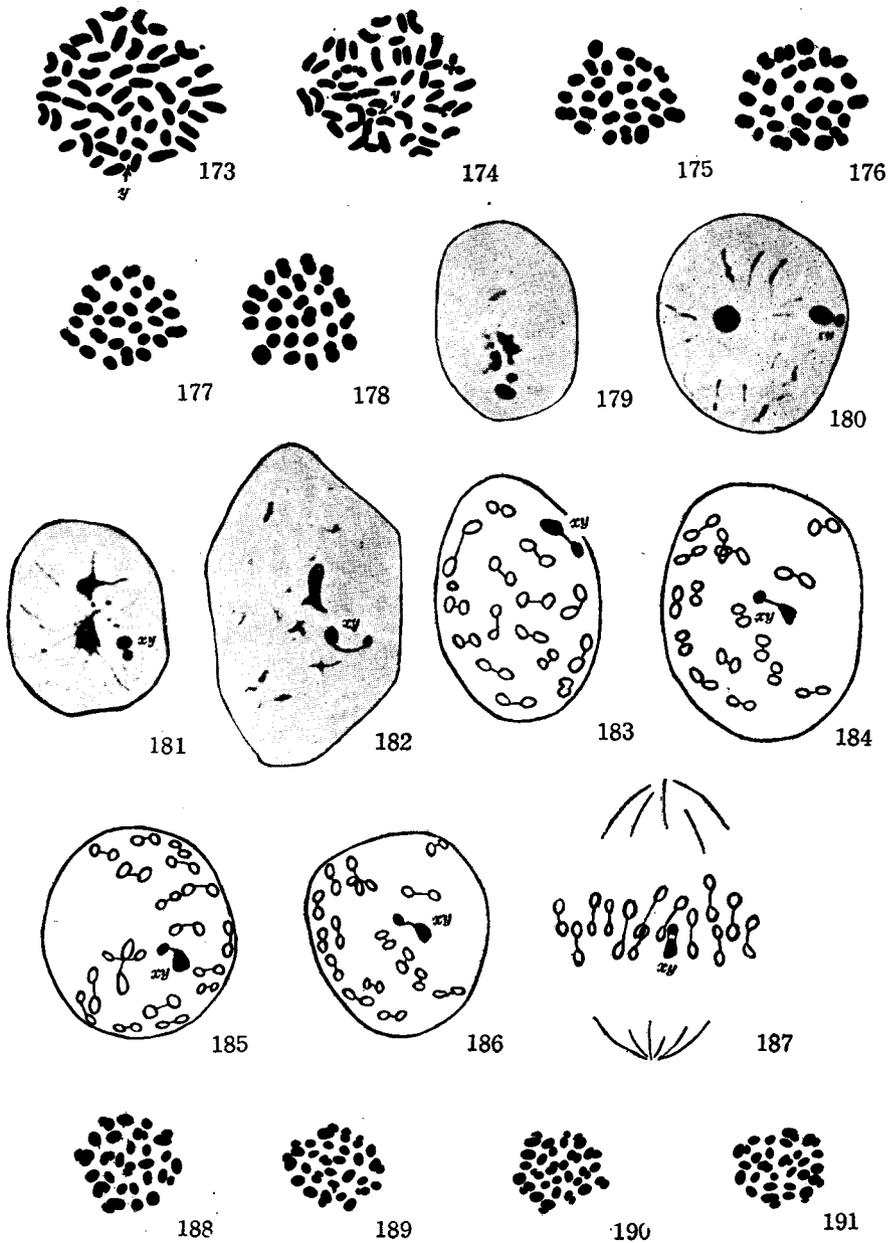
### PLATE XIII

- Figs. 163–172. Chromosomes of *Tecticeps japonicus*.
- 163–164. Metaphase chromosomes of spermatogonia, 63 (27V' + 36R') autosomes and an X chromosome in each.
    - 165. Serial alignment of the paired chromosomes from Fig. 163.
    - 166. Nucleus in diakinesis stage, indicating an X chromosome in deep black.
  - 167–168. Polar views of metaphase chromosomes of primary spermatocytes, 31 bivalents and an X-chromosome in each.
    - 169. Metaphase chromosomes of secondary spermatocyte containing an X-chromosome.
  - 170–171. Side views of metaphase chromosomes of primary spermatocytes.
    - 172. Side view of early anaphase chromosomes of primary spermatocyte.



## PLATE XIV

- Figs. 173–191. Chromosomes of *Anisogammarus annandalei*.
- 173–174. Polar views of spermatogonial metaphase, 54 chromosomes in each. Y-chromosome ( $y$ ) is indicated as the smallest one.
- 175–178. Polar views of metaphase chromosomes of primary spermatocyte, 27 bivalents in each.
- 179–182. Leptotene nuclei of primary spermatocytes, indicating two chromosome nucleoli ( $xy$ ).
- 183–186. Four nuclei in diakinesis stage, indicating XY complexes ( $xy$ ) in deep black.
187. Side view of metaphase chromosomes of primary spermatocyte, sex-chromosomes ( $xy$ ) is indicated in deep black.
- 188–191. Polar view of metaphase chromosomes of secondary spermatocytes, 27 dyads in each.



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