A CHROMOSOME STUDY IN FIVE SPECIES OF ISOPOD CRUSTACEA

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In reference to the literature pertaining to the chromosome study of the isopod Crustacea, there are accessible several papers dealing with the chromosomes of 28 species which belong to five suborders of the Isopods: the chromosomes of 15 species of the Oniscoidea were reported by Nichols '01, '02, '09, Mir '38, Radu '30, '31, Vandel '26, '28 ,34, '41 and Imai & Makino '40, those of 7 species of Asellota by Carnoy '85, Sugiyama '33, Dworak '35, Vandel '38, '41, Staiger & Bocquet '54 and Vitagliano '47, those of one species of Valvifera by Nichols '09, those of one species of Epicaridea by Hiraiwa '36, and those of two species of Flabelifera by Callan '40 and Niiyama '56. In his review on the chromosomes of isopods Vandel ('41) stated that the marine isopods and the related terrestrial and fresh-water species are characterized by the haploid number of 28, or thereabouts. Some information on the existence of the sex-determining chromosome in isopods has been communicated by Dworak '35, Staiger & Bocquet '54 and Niiyama '56.

The present paper describes the results of a chromosome study in five species of the isopod Crustacea of which two species are classified into suborder Valvifera, one into Flabellifera and two into Oniscoidea. The chromosome number here determined for the above five species differs from the number reported in many related species by Vandel ('41).

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Material and Method

The following five species of isopods: *Cleantiella isops* (GRUBE), *Idotea japonica* RICHARDSON, *Cymodoce japonicus* RICHARDSON. *Megaligia exotica* (ROUX) and *Tylos granulata* MIERS, came under investigation in the present study. Generally the testes of isopods consist of three pairs of testicular lobes, each having a spindle shape and being connected by a pair of the vas deferens. They were removed by vivisection and fixed in the modified weak Flmming's mixture.

Observations

1) *Cleantiella isops* (GRUBE)

This species is a member of the suborder Valvifera showing a wide distribution in the sea-shore throughout Japan. It measures 20-30 mm 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. The three pairs of testicular lobes do not show the same stage in process of maturation. The anterior lobe contains spermatogonial cells, the middle lobe is provided with spermatocytes of different stages while the posterior lobe is filled with mature spermatozoa.

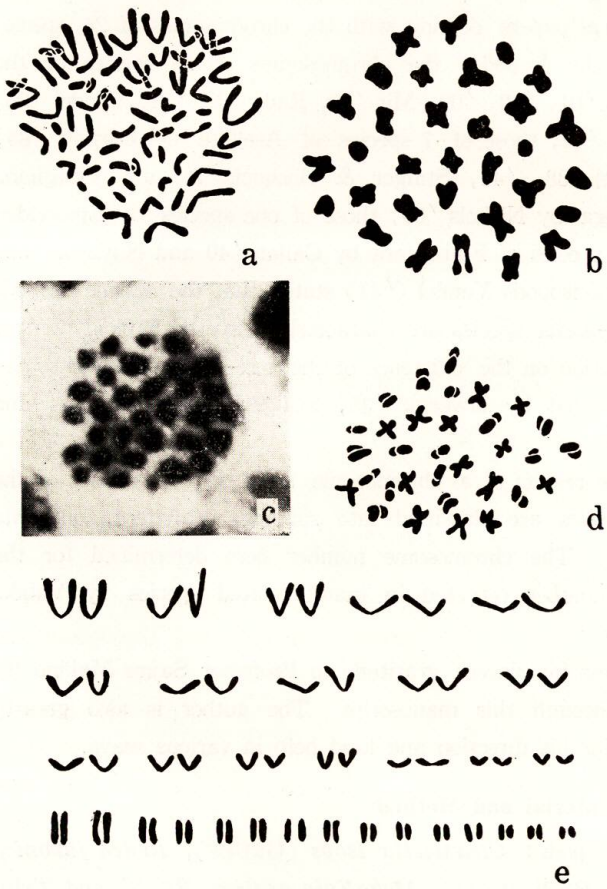

Fig. 1. Chromosomes of *Cleantiella isops* (GRUBE)
a. Spermatogonial metaphase, 64 chromosomes. ×3700 b. Metaphase of primary spermatocyte, 32 tetrads. ×3700 c. Microphotograph of Fig. b. ×2400 d. Metaphase of secondary spermatocyte, 32 dyads. ×3700 e. Serial alignment of the paired chromosomes from Fig. a.

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. 1, a. Karyogram was analysed and shown Fig. 1, e by paired alignment of supposed homologous chromosomse. It is evident that the diploid complex is composed of 17 pairs of metacentric V-shaped elements and 15 pairs of acrocentric ones of rod-and dot-type. All elements can be paired in the diploid complex. Many ¡dividing figures were observed n the primary and secondary spermatocytes. There are observable 32 bivalents in every metaphase plate of the primary spermatocyte (Fig. 1, b-c). The metaphase polar view of the secondary spermatocyte shows 32 chromosomes of a dyad. nature (Fig. 1, d). The chromosome number of the species is therefore 64 in diploid and 32 in haploid. There are no chromosomes which show form and behaviour characteristic to the sex-chromosome.

2) *Idotea japonica* RICHARDSON

This species is also a member of the suborder Valvifera : individuals are 25-45 mm in body length. They are common sea-shore animals in northern parts of Japan. The specimens for the present study were collected abundantly in the same place and simul-

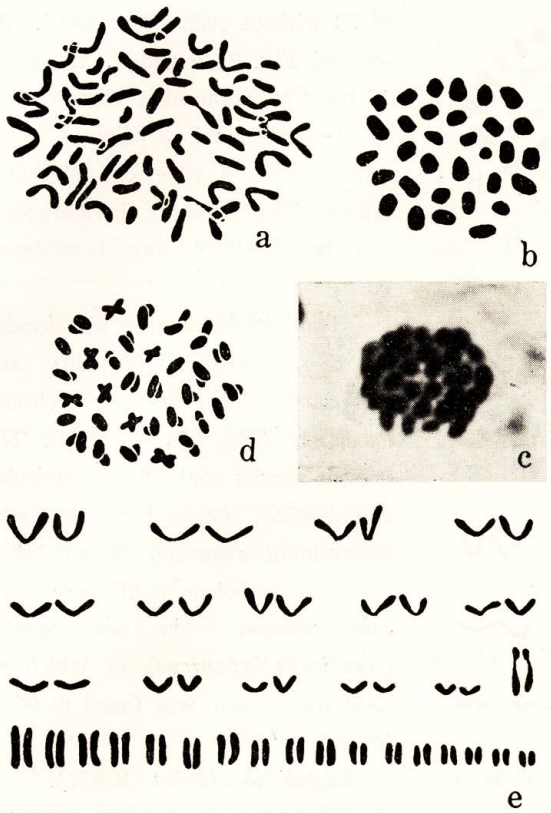

Fig. 2. Chromosomes of *Idotea japonica* RICHARDSON
a. Spermatogonial metaphase, 64 chromosomes. ×3700 b. Metaphase of primary spermatocyte, 32 tetrads. ×3700 c. Microphotograph of Fig. b. ×2400 d. Metaphase of secondary spermatocyte, 32 dyads. ×3700 e. Serial alignment of the paired chromosomes from Fig. a.

taneously with the previous species.

The diploid number obsreved in the spermatogonium was decided as 64 (Fig. 2, a). The diploid complex is composed of 15 pairs of metacentric elements and 17 pairs of acrocentric ones varying in shape from rod to dot. One pair ammongst the metacentric chromosomes are satelite-like in structure, since each of them consists of a small dot-like body and a long rod-like one connected therewith by means of a fine thread. The elements forming the remaining 14 pairs are of V-shape. The haploid number 32 was determined through observations of the primary and secondary spermatocytes at metaphase (Fig. 2, b,c,d). No chromosome with any peculiar characters could be detected throughout the two reduction divisions.

The present species is identical in chromosome number to the previously described species (*Cleantiella isops*), but differs in chromosome costitution; namely, the previous species has four more metacentric chromosomes and four less acrocentric ones than the present species. The presence of a pair of satellite-like chromosomes is also a characteristic feature of the present species.

3) *Cymodoce japonicus* RICHARDSON

This species is a member of the suborder Flabellifera and belongs to the subfamily Sphaerominae in one species (*Tecticeps japonicus*) of which the present author reported the occurrence of an XO sex-mechanism in males (Niiyama '56). It is 10-25 mm in body length. The specimens for this study were collected from holes in wooden piers made by shipworms, *Teredo*, along the sea-shore of the Akkeshi Marine Biological Station, in September, 1944.

Careful counting of excellent spermatogonial metaphase plates, gave the diploid number

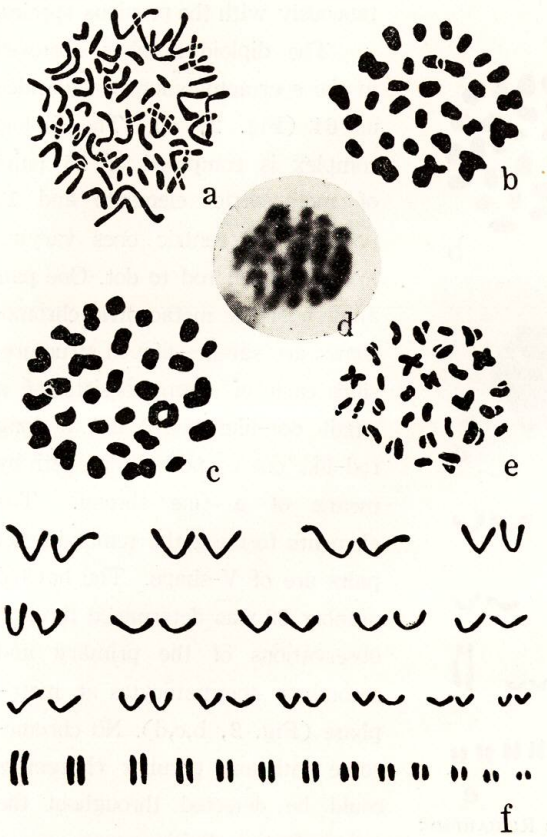

Fig. 3. Chromosomes of *Cymodoce japonica* RICHARDSON
a. Spermatogonial metaphase, 58 chromosomes. ×3700
b-c. Metaphase of primary spermatocyte, 29 tetrads. ×3700 d. Microphotograph of Fig. b. ×2400 e. Metaphase of secondary spermatocyte, 29 dyads. ×3700
f. Serial alignment of the paired chromosomes from Fig. a.

of 58 without even slight doubt. As seen in Fig. 3, a and f, there are 16 pairs of metacentric chromosomes of V-shaped and 13 pairs of acrocentric ones of rod- or dot-shape. All the chromosomes of spermatogonia can be arranged into homologous pairs.

The haploid number was decided as 29 in both the primary and secondary spermatocytes without exception (Fig. 3, b. c, d, e). The present species contains no particular chromosome regarded as the sex-determining elements, though it is classified to belong to the subfamily Sphaerominae, in one species (*Tecticeps japonicus*) of which an X-O mechanism was found to occur (Niiyama '56).

4) *Megaligia exotica* (ROUX)

This species belongs to the suborder Oniscoidea. It is one of the very common sea-side isopods found all around Japan. Specimens capture on rocky seaside places in the vicinity of Oshoro near Otaru, in June, 1954 provided the material for study. Body length of the species is 30-45 mm.

The diploid number 72 was decided through the observations of several good spermatogonial metaphase plates. As seen in Fig. 4, a and f, six pairs of metacentric V-shaped chromosomes and 30 pairs of acrocentric chromosomes, rod- and dot-like in form constitute the spermatogonial complex. The metaphase of the primary spermatocyte invariably showed 36 bivalents of various form (Fig. 4, b,c,d). As seen in Fig. 4, e, the same number was observable in every equatorial plate of the secondary spermatocyte. All the elements divide synchronously at anaphase of each both first and second divisions, without showing any element of unusual behaviour.

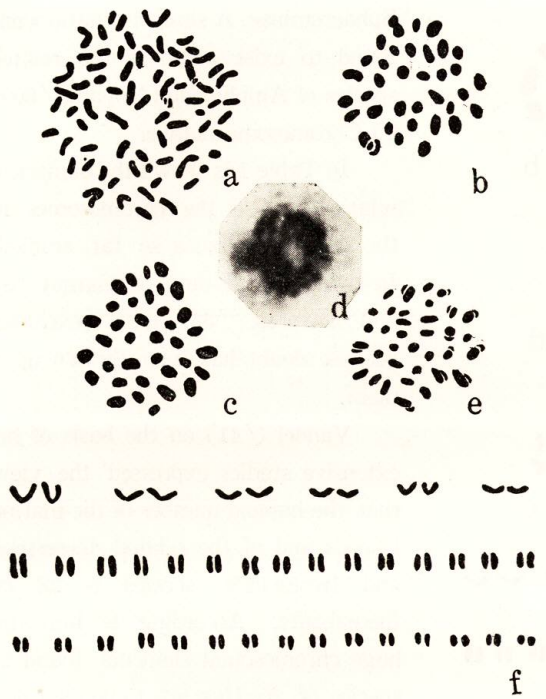

Fig. 4. Chromosomes of *Megaligia exotica* (Roux) a. Spermatogonial metaphase, 72 chromosomes. ×3700 b-c. Metaphase of primary spermatocyte, 36 tetrads. ×3700 d. Microphotograph of Fig. b. ×2400 e. Metaphase of secondary spermatocyte, 36 dyads. ×3700 f. Serial alignment of the paired chromosomes from Fig. a.

5) *Tylos granulata* Miers

This species together with the previous species is a member of the suborder Oniscoidea. It is a terrestrial isopod of 10 mm in body length. They abound in sandy sea-shores throughout Japan. The present study was based on testicular material derived from several specimens captured in Ranshima, near Otaru in June, 1950.

It was found after careful examination of several spermatogonial metaphases that 68 chromosomes constituted the male diploid complex of this species (Fig. 5, a). The complex consists of 10 pairs of metacentric chromosomes and 24 paire of achrocentric ones (Fig. 5, f). Every metaphase plate of both primary and secondary spermatocytes showed 34 chromosomes with indisputable clearness (Fig. 5, b, c, d, e). No chromosome, peculiar in shape and behaviour, was found in either of the meiotic divisions.

Discussion

1) Sex-chromosome

On reviewing the literature of the chromosomes of isopods, one finds useful three papers which deal with the presence of the sex-chromosome. Dworak ('35) reported in *Asellus aquaticus* an XO sex-mechanism in the male, but her results were found by Vitagliano ('47) and Niiyama ('56) to be probably erroneous. The argument of Staiger and Bocquet ('54) on the occurrence of female hetrogamety in four closely related subspecies of *Jaera marina* (Janiridlae, Asellota) is not fully conclusive as discussed in the previous paper of the present author (Niiyama '56). An XO sex-mechanism was found to occur in males of *Tecticeps japonicus* (Sphaerominae, Sphaeromidae, Flabellifera) by the author's previous study (Niiyama '56). As described in the foregoing pages, *Cymodoce japonica* showed no indication of the presence of any particular chromosome although it and *Tecticeps japonicus* which latter has an X-O mechanism are members of the subfamily

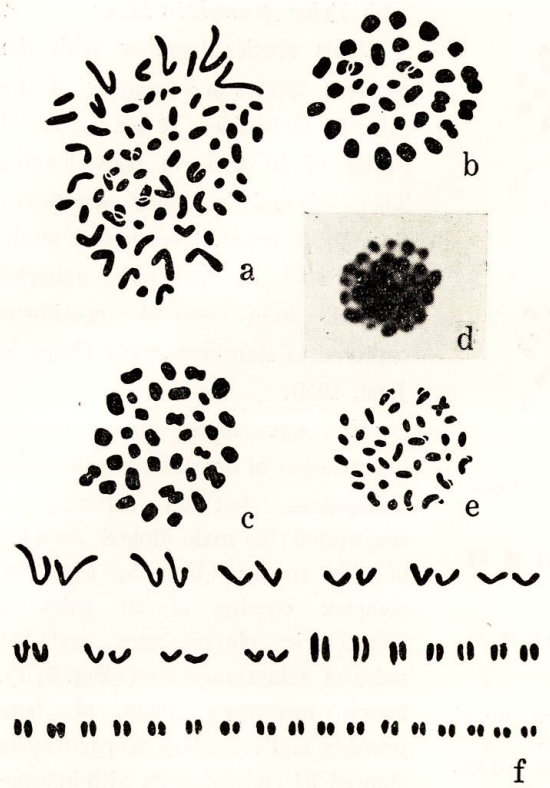

Fig. 5. Chromosomes of *Tylos granulata* MIERS
a. Spematogonial metaphase. 68 chromosomes. ×3700
b-c. Metaphase of primary spermatocyte, 34 tetrads. ×3700 d. Microphotograph of Fig. b. ×2400 e Metaphase of secondary spermatocyte, 34 dyads. ×3700
f. Serial alignment of the paired chromosomes from Fig. a.

Sphaerominae. A similar situatio wnas found to exist amongst the related species of Amphipoda (Niiyama '50).

2) Chromosome number

In Table 1 is shown the numerical relationship of the chromosomes in the isopod Crustacea so far studied. In the table the data of Carnoy '85 and Dworak '35 were excluded because doubt has been thrown upon them.

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 freshwater 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*, thogh there is no positive proof for the above consideration. Vandel states that the chromosome number of the true Oniscoidea shows a tendency to increase with the evolution of species: the following relationship seemes to occur in the chromosome number of isopods.

Marine species (n,28)—{Asellus (n,8)—Oniscidae (n,16 to 24)—{Porcellio (n,28)
{Trichoniscus {Armadillidium

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

By reference to Table 1, it is evident that among marine species, *Cleantiella isops*, *Idotea japonica*, *Cymodoce japonicus* and *Tecticeps japonicus* have a high chromosome number, while *Jaera marina* shows a number lower than 28. Certain terrestrial species, also, have a high number of chromosome: for instance, n, 31 for *Ligidium hypnorum*; n, 30 for *Ligia oceanica*; n, 34 for *Tylos granulata* and n, 36 for *Megaligia exotica* are available for reference. In the author's opinion the relationship between the chromosome

Table 1. List of the chromosome number of the Order Isopoda

Species	2*n*	*n*	Author
Suborder Oniscoidea			
Family Oniscidae			
Sufamily Armadillidiinae			
Armadillidium opacum	54 s	27 ♂	Radu '30
Armadillidium nasatum		28 ♀ I	Vandel '41
Armadillidium vulgare	54 s	27 ♂ I	Radu '31
Armaillidium vulgare		28 ♀ I	Vandel '41
Subfamily Oniscinae			
Oniscus asellus	32 s	16 ♂ ♀	Nichols '01, '02, '04
Chaetophiloscia elongata		24 ♀ I	Vandel '41
Philoscia gallicus		24 ♀ I	Vandel '41
Subfamily Porcellioninae			
Porcellio scaber	56 s	28 ♂ I,II	Imai & Makino '40
Porcellio scaber		28 ♀ I	Vandel '41
Porcellio loevis		28 ♀ I	Vandel '41
Porcellio gallicus		28 ♀ I	Vandel '41
Porcellio muscorum		24 ♀ I	Vandel '41
Family Ligiidae			
Ligia italica		28 ♂ ♀	Mir '39
Ligia oceanica		30 ♂	Mir '39
Ligidium hypnorum		31 ♂	Mir '39
Megaligia exotica	72 s	36 ♂ I,II	This paper
Family Tyliidae			
Tylos granulata	68 s	34 ♂ I,II	This paper
Family Trichoniscidae			
Trichoniscus biformatus	16 s	8 ♂	Vandel '26, '28, '34
Trichoniscus elisabethae	16 s	8 ♂	Vandel '26, '28, '34
Trichoniscus provisorum	16 s	8 ♂	Vandel '26, '28, '34
Suborder Asellota			
Family Asellidae			
Asellus aquaticus	16 s	8 ♂ I	Vandel '38, '41; Vitagliano '47
Asellus (*Proasellus*) *meridianus*	16 s	8 ♂ I	Vandel '38, '41
Asellus nipponensis	14 s	7 ♂ I	Sugiyama '33
Stenasellus virei		27 ♂	Vandel '38 '41
Family Janiridae			
Jaera marina forsmani		8+XY_1Y_2 ♀ I	Staiger & Bocquet '54
Jaera marina ischiosetosa		12+XY_1Y_2 ♀ I	Staiger & Bocquet '54
Suborder Valvifera			
Family Idoteidae			
Cleantiella isops	64 s	32 ♂ I,II	This paper
Idotea irrorata		28 ♂	Nichols '09
Idotea japonica	64 s	32 ♂ I,II	This paper
Suborder Epicaridea			
Family Bopyridae			
Epipenaeon japonica	1€cl	8p.b.	Hiraiwa '36
Suborder Flabellifera			
Family Cymothoidae			
Anilocra mediterranea	12s,o	6 ♂	Callan '40
Family Sphaeromidae			
Cymodoce japonica	58 s	29 ♂ I,II	This paper
Tecticeps japonicus	63 s	31+X ♂ I 31,32 ♂ II	Niiyama '56

S: spermatogonium O: oogonium cl: cleavage p.b.: polar body
I: primary spermatocyte II: secondary spermatocyte

number and the taxonomical situation in the isopod Crustacea is difficult to establish in the present status of investigation, and sufficient information needful for decision can not be expected until after the chromosomes of many species have been exhaustively investigated. The data on the chromosomes of the isopod Crustacea is 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 proof upon which to enter into a consideration on the reduction of the chromosome number as a result of chromosome fusion. To attempt to formulate any rule for general application, it is necessary to accumulate cytological data on a very large number of species.

Summary

The chromosomes of the following five species of isopod Crustacea were studied in their male germ cells. The number and the formula of chromosomes for each species are shown as follows:

Species	2n	n
Cleantiella isops	64=34V's + 30R's	32
Idotea japonica	64=30V's + 34R's	32
Cymodoce japonicus	58=32V's + 26R's	29
Megaligia exotica	72=12V's + 60R's	36
Tylos granulata	68=20V' + 48R's	34

V: metacentric chromosome R: rod- or dot-shaped acrocentric chromosome

In all species here studied, there is no chromosome, having characteristic usual to the sex-determining chromosome in form and behaviour.

Literature cited

Carnoy, J. B. (1885). La cytodiérèse chez les Arthropodes. *La Cellule* 1.

Callan, H. G. (1940). The chromosomes of the cymothoid isopod, Anilocra. *Q. J. M. S.* 52.

Dworak, I. (1935). Maturation des cellules sexuelles mâles et structures, cytoplasmiques dans la spermatogénèse chez 1'*Asellus aquaictus*. *Folia Morphologica* V.

Hiraiwa, K. (1936). Studies on a bopyrid, *Epipenaeon japonica* THIEL III. Development and life-cycle, with special reference to the sex differentiation in the bopyrid. *Journ. Sci. Hiroshima Univ. Ser. B* Div. I. Art. 8.

Imai, G. & Makino, S. (1940). The chromosomes of *Porcellio scaber* LATREILLE (Isopoda, Oniscidae). *Jap. Journ. Genet.* 16.

Mir, C. (1939). Le nomdre de chromosomes des Ligiidae. *C.R.S.A.Sci.* 209.

Nichols, M. L. (1909). Comparative studies in crustacean chromosomes. *Journ. Morph.* 20.

Niiyama, H. (1950). The X-Y mechanism of the sex-chromosome in the male of *Anisogammarus annandalei* (TATTERSALL) (Crustacea: Amphipoda). *Annot. Zool. Japon.* 23.

———— (1956). Cytological demonstration of an XO sex-mechanism in males of *Tecticeps japonicus*,

an isopod Crustacea. *Cytologia* 21.

Radu, V. (1930). The spermatogénèse chez *Armadillidium opacum*. *C.R.S.B.* 105.

———— (1931). Spermatogeneza la *Armadillidium vulgare* LATR., cu privire spciala asupra evolutiei chromatinei si a constituantilor cytoplasmatici. *Mem. Sect. Stiintif. Acad. Romania, Ser.* III, VIII.

Staiger, H. & Bocquet, Ch. (1954). Cytological demonstration of female heterogamety in Isopods. *Experientia* X/2.

Vitagliano, G. (1947). La spermatogenesi e la distribuzione dei chiasmi in *Asellus aquaticus* L. *Pubbl. della Staz. Zool. Napoli* XXI.

Vandel, M. A. (1926). Triploidie et parthénogenèse chez 1'Isopode, *Trichoniscus* (*Spiloniscus*) *provisorius* ROCOVITZA *C.R.A.S.* 183.

———— (1928). La parthénogenèse géographique. Contribution â 1'étude biologique et cytologique de la parthénogenèse de naturelle. *Bull. Biol. Fra. Belg.* 62.

———— (1934). La parthénogenèse geographique. II. Les mâles triploides d'origine parthénogenètique de *Trichoniscus elisabethae* HEROLD *Bull. Biol. Fra. Belg.* 68.

———— (1938). Les chromosomes des Asellotes. *C.R.S.A.Sci.* 206.

———— (1941). Etude des garnitures chromosomiques de quelques Crustaces isopodes terrestres et d'eandonce europeens *Cytologia* 12.