

# GENEN EN PHAENEN

Tijdschrift van de Nederlandse Genetische Vereniging

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Administratie: Federatie van Medisch-Biologische Verenigingen in Nederland,  
Geert Grooteplein Noord 21, Nijmegen

Leden van de Ned. Gen. Ver. ontvangen het tijdschrift gratis.

Contributie f 20.- per jaar voor gewone leden, f 5.- voor student-leden. Penningmeester Drs. A. A. J. van der Leun,  
Rijksvoorlichtingsdienst voor de Pluimveeteelt, Utrechtseweg 31, Zeist, tel. 03404/16911. Giro 43150

## REVIEW OF THE GERM CELL CHROMOSOME CYTOLOGY OF COLLEMBOLA, WITH A LIST OF CHROMOSOME NUMBERS AND DATA ON TWO SPECIES NEW TO CYTOLOGY

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Though still greatly incomplete, the knowledge on the cytotaxonomy of springtails has increased essentially in recent years. In view of the primitive phylogenetic origin of the group (of. SHAROV, 1966) and, consequently, its general importance for cytophylogenetic problems, it seems useful to summarize briefly the evidence hitherto available.

In the present paper, for reason of convenience, the system of PACLT (1956) is used. Recent studies on the morphology and ontogeny of the Apterygota and Pterygota, however, have produced a strong evidence on the distinct paths of evolution of Protura, Collembola, Diplura and Insecta s. str. These observations make necessary to elevate the former three apterygotan orders to the rank of separate classes, whereas the class Insecta should contain the Thysanura and Pterygota only (SHAROV, 1966).

So far 46 species and subspecies of both suborders belonging to seven of the eleven living collembole families were studied cytologically (all after the classification of PACLT, 1956). The chromosome numbers of *Folsomia candida distincta* Bagnall (Fig. 1) and *Allacma fusca* (L.) (Fig. 6) are for the first time reported in this paper, and so are the spermatogonial

Table I

## Synopsis of the main cytotaxonomic data on Collembola

SUBORDER	Family (Subfamily)	Locality	Chromosome number				Sex chromosomes and sex determination	Remarks	References	
			2n		n					
			♂	♀	♂	♀				
ARTHROPLEONA Poduromorpha	Poduridae	<del>Podura aquatica L.</del>	Belgium	8				WILLEM, 1900		
	Hypogastruridae	<del>Cerastophysella armata communis (Fols.)</del>	Argentina			7		occasionally 1-3 supernumerary elements	NUNEZ, 1962	
		<del>Hypogastrura mansuetalis (Tullb.)</del>	Argentina	14		7		occasionally 1-3 supernumerary elements	NUNEZ, 1962	
		<del>Hypogastrura viatica (Tullb.)</del>	Finland			7			SAURE & BRUMMER- KORVENKONTIO, 1958	
	Neanuridae (Brachystomel- linae)	<del>Brachystomella parvula (Schiff.)</del>	Argentina	8		4			NUNEZ, 1962	
		<del>Probrachystomella rhodocoma Rap.</del>	Argentina			6			NUNEZ, 1962	
	(Pseudochoru- tinae)	<del>Anurida maritima (Guérin)</del>	U.S.A.	8				chromosome number given as "usually"	CLAYPOLE, 1896	
	(Neanurinae)	<del>Bilobella massoudi Cassagnau</del>	Slovenia (Yugoslavia)					2n = 14 in salivary glands	CASSAGNAU, 1968	
	Estomochyromorpha	Isotomidae (Protoisotominae)	<del>Balistura scoetti (Dalla Torre)</del>	Argentina			7			NUNEZ, 1962; 1968
			<del>Protoisotoma lespanii Rap.</del>	Argentina			7			NUNEZ, 1962
			<del>Protoisotoma minutus (Tullb.)</del>	Argentina			4			NUNEZ, 1962
			<del>Folsomia candida distincta Bagnall</del>	Netherlands	13?		7		probably partheno- genetic	this paper
			<del>Folsomia sexoculata (Tullb.)</del>	Finland			7			SAURE & BRUMMER- KORVENKONTIO, 1958
		(Isotominae)	<del>Isotoma antennalis (Bagnall)</del>	Argentina			7		X recognizable	NUNEZ, 1968
			<del>Isotoma maritima Tullb.</del>	Finland			7			SAURE & BRUMMER- KORVENKONTIO, 1958
			<del>Isotoma sensibilis Tullb.</del>	Finland			7			SAURE & BRUMMER- KORVENKONTIO, 1958
			<del>Isotoma viridis Bourl.</del>	Finland			7			SAURE & BRUMMER- KORVENKONTIO, 1958
			<del>Isotomina thetophila (Azels.)</del>	Argentina			5			NUNEZ, 1962
			<del>Isotomurus valurris (Müller)</del>	Finland			7		at ♀ diakinesis 6 bivalents and one minute, presumably univalent "accessory chromosome"	SAURE & BRUMMER- KORVENKONTIO, 1958
			Argentina			7			NUNEZ, 1962	
Tomoceridae		<del>Pogonognathellus flavescens (Tullb.)</del>	Finland			6		sub Tomocerus: at ♀ I, one trivalent	SAURE & BRUMMER- KORVENKONTIO, 1958	
		<del>Pogonognathellus longicornis (Müller)</del>	Finland			6		sub Tomocerus: at ♀ I, one trivalent	SAURE & BRUMMER- KORVENKONTIO, 1958	

	<i>Tomocerus minor</i> (Lubb.)	Finland				6		at ♀ I, one trivalent	SAURE & BRUMMER-KORVENKONTIO, 1958
		Netherlands	11			6	6	at I, one element extra large	this paper
	<i>Tomocerus minutus</i> Tullb.	Japan				6		at ♀ I, all elements similar in size; no trivalent structure	SAITOH & CHIBA, 1959
	<i>Tomocerus vulgaris</i> (Tullb.)	Finland				6		at ♀ I, one trivalent	SAURE & BRUMMER-KORVENKONTIO, 1958
Entomobryidae (Entomobryinae)	<i>Entomobrya atrocincta pseudoperpulchra</i> Mills	Argentina	11			6	XO/XX		NUNEZ, 1962
	<i>Entomobrya corticalis</i> (Nic.)	Finland				6			SAURE & BRUMMER-KORVENKONTIO, 1958
	<i>Entomobrya lanuginosa</i> (Nic.)	Finland				6			SAURE & BRUMMER-KORVENKONTIO, 1958
	<i>Entomobrya lanuginosa olivacea</i> Rap.	Argentina	11	12		6	XO/XX		NUNEZ, 1962
	<i>Entomobrya multifasciata</i> (Tullb.)	Argentina	11			6	XO/XX		NUNEZ, 1962; 1968
	<i>Entomobrya cf. nigrocincta</i> Denis	France	?	?			X not recognizable	minute elements, chromosome number not identified	TUZET & MANIER, 1956
	<i>Entomobrya cf. nivalis</i> (L.)	France	9		5		XO/XX; pre-reduction of sex element		TUZET & MANIER, 1956
	<i>Entomobrya nivalis</i> (L.)	Finland				6			SAURE & BRUMMER-KORVENKONTIO, 1958
	<i>Entomobrya pseudodecora</i> Rap.	Argentina	11	12		6	XO/XX	in one ♂ 2n = 12 and in another one 2n = 14; at early prophase one element heteropycnotic	NUNEZ, 1962
	<i>Pseudosigella sexculata</i> Schött.	Argentina				6	XO/XX		NUNEZ, 1962
	<i>Seira domestica</i> (Nic.)	Argentina		12		6	XO/XX	sub <i>Lepidocyrtinus</i>	NUNEZ, 1962
(Orchesellinae)	<i>Orchesella bifasciata</i> Nic.	Finland				6			SAURE & BRUMMER-KORVENKONTIO, 1958
	<i>Orchesella villosa</i> (Geoffr.)	France				6?			LÉCAILLON, 1901
		France	9		5		XO/XX; pre-reduction of sex element		TUZET & MANIER, 1956
(Cyphoderinae)	<i>Cyphoderus assimilis empodialis</i> Rap.	Argentina				6	XO/XX		NUNEZ, 1962
SYMPHYLEONA									
Sminthuridae (Sminthuridinae)	<i>Sminthurides aquaticus</i> (Bourl.)	Finland				5			SAURE & BRUMMER-KORVENKONTIO, 1958
	<i>Sphaeridia pumilis</i> (Krausb.)	Argentina		10		5	XO/XX		NUNEZ, 1962
(Katianninae)	<i>Katianna</i> sp.	Argentina	9			5	XO/XX		NUNEZ, 1962
(Sminthurinae)	<i>Allacma fusca</i> (L.)	Netherlands		12?		6?			this paper
	<i>Sminthurinus thomei</i> Börner	Argentina				5	XO/XX		NUNEZ, 1962
	<i>Sminthurinus thomei</i> Rap.	Argentina				5	XO/XX		NUNEZ, 1962
	<i>Sminthurus viridis</i> L.	France	9		5		XO/XX (?), pre-reduction of sex element		TUZET & MANIER, 1956
(Dicyrtominae)	<i>Dicyrtomina</i> sp.	Argentina	9			5	XO/XX		NUNEZ, 1962

and spermatocyte numbers of *Tomocerus minor* (Lubb.) (Figs. 2-5). All three species originate from various localities in the Netherlands. (For further locality data see caption of Figs. 1-6).



Figs. 1-6. Germ cell chromosomes of some Collembola (acetic orcein squash, 1760 x) : 1. *Folsomia candida distincta* Baghall, 1936 (Utrecht, III-X-1968); primary oocyte prometaphase; - 2 - 5. *Tomocerus minor* (Lubbock, 1862) (Baarnse bos, prov. Utrecht, 11-IX-1968, 6-X-1968): 2. spermatogonial prometaphase, 3. secondary oocyte metaphase, 4. spermatocyte diakinesis, 5. primary spermatocyte metaphase; - 6. *Allacma fusca* (Linnaeus, 1758) (Baarnse bos, prov. Utrecht, 6-X-1968) : primary oocyte prometaphase.

In Table I a synopsis is given of the main cytotaxonomic data of the order.

In the following a few special topics of collembole cytotaxonomy will be discussed in some detail.

### I. Chromosome number

Among 46 cytologically studied forms, there was only one species in which the chromosome number could not be identified, though in several others the counts are uncertain. The haploid numbers are known in 43 species. In most of these (40) only females have been studied. Since little is known on sex determination in the order, the distribution of the oocyte chromosome numbers only can be further considered.

The female haploid chromosome number in Collembola varies between  $n = 4$  and  $n = 7$ . The few male complements described also fall within these limits. A histogram of the order is given in Fig. 7.

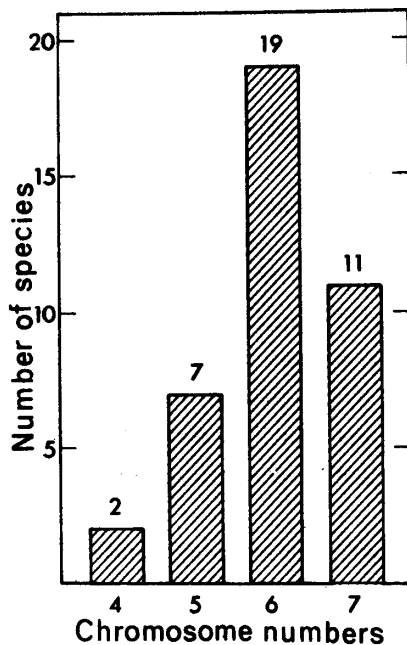


Fig. 7. Histogram of the female haploid chromosome numbers in Collembola

It is evident, from Fig. 7, that the haploid number,  $n = 6$ , is the most common in the order (19 species); nevertheless, it can probably not be regarded as the true type number of Collembola. The problem becomes apparent when the numeric variation is reviewed from the point of view of the distribution within the higher taxa. This has been done in Table II.

Table II

Distribution of the female haploid chromosome numbers and family type numbers in Collembola

Suborder and family	Number of species studied	Number of species with haploid chromosome number				Family type number
		4	5	6	7	
<b>ARTHROPLEONA</b>						
Poduridae	1	?				?
Hypogastruridae	3				3	7
Neanuridae	2	1		1		?
Isotomidae	10	1	1		8	7
Tomoceridae	5			5		6
Entomobryidae	12			12		6
<b>SYMPHYPLEONA</b>						
Sminthuridae	7		6	1		5

As far as the numeric variation of the karyotype is concerned, the two suborders differ essentially.

In Arthropleona the chromosome numbers in different families vary between  $n = 4$  and  $n = 7$ . Due to the pronounced variation in the family type numbers, the actual type number of the suborder is obscure.

There is no numeric variation in the oocyte complements of the studied representatives of the families *Hypogastruridae* ( $n = 7$ ), *Tomoceridae* and *Entomobryidae* (both  $n = 6$ ), though in *Tomoceridae* the exceptionally big element found in most of the species studied might well originate in a fusion of two smaller elements of a "primary" karyotype.

Nothing can be said as to the type number of *Neanuridae*. From the cytological point of view the family appears to combine a conglomerate of heterogeneous forms.

Less complicated is the situation in *Isotomidae*. The family type number definitely is  $n = 7$ , though in each of the two subfamilies studied there is one species deviating from the general pattern.

Since the family type number can be identified with certainty in only four families of the suborder, it might well be possible that the quantitative prevalence of the  $n = 6$  complements is due solely to the relatively high number of representatives of *Tomoceridae* and *Entomobryidae* studied cytologically. The possibility of a secondary character of the tomoceridan complement all the more calls for prudence in making any assumptions at the present state of our knowledge.

Contrary to Arthropleona, the complements of Symphypleona are numerically uniform. The type number,  $n = 5$ , was found in all but one species of the four subfamilies studied, though also in *Allacma fusca* (L.) several primary and secondary oocytes were found in our material with presumably 5 elements only.

From this record it is evident that whether the type number of Arthropleona be 6 or 7, the Symphypleona clearly differ from the former by their lower chromosome number.

## II. Morphology and behaviour of the germ cell chromosomes

The mitotic elements are uniform in shape and only very slightly decreasing in size. In lacto-aceto-orcein/squash preparations their length usually varies between 1-2  $\mu$  (*Folsomia candida*). Due to the minute size no infra-structures were so far observed.

The elements in the polar views of meiotic metaphase usually exhibit greater variations in size and can often be distinguished by their shape.

At mitotic metaphase the elements tend to clump together. The best results in spreading them in the orcein squashes were obtained by the application of a pressure of  $\pm 700 \text{ kg/cm}^2$  on the cover slip (for the original equipment used cf. MONTIJN, 1970).

At spermatocyte pachytene the elements are organised in a bouquet (cf. also SAURE & BRUMMER-KORVENKONTIO, 1958). At least in *Folsomia candida distincta* Bagnall and *Tomocerus minor* (Lubb.) the bouquet is present also in the oocytes.

At diakinesis the elements appear as cross and ring bivalents. Their bivalent structure is recognizable in bigger chromosomes even at the maximal contraction of oocyte and spermatocyte metaphase.

In *Tomocerus minor* (Lubb.) at primary oocyte anaphase the elements divide equationally and simultaneously, though in some figures lagging and bridges were observed.

The second meiotic cycle follows the first without a clear interkinetic stage (SAURE & BRUMMER-KORVENKONTIO, 1958). Bridges are a regular feature of second spermatocyte anaphase. They are caused by the fact that the distal ends of the longest elements remain in contact when the other chromosomes have already moved apart (NUNEZ, 1962).

## III. Sex chromosomes and sex determination

For most collembolan families little can be said on sex chromosomes and sex determination.

The XO/XX sex determination, with the male being the heterogametic sex, is almost certain for the families *Entomobryidae* and *Sminthuridae* (cf. NUNEZ, 1962). The numeric situation in the complement of *Tomocerus minor* (Lubb.) may perhaps also suggest a similar type of sex determination in

this species.

Aside from these cases the sex element can be recognized with certainty only in the primary spermatocyte anaphase of *Isotoma antennalis* (Bag-nall) (NUNEZ, 1968, fig.).

In oocyte diakinesis of *Isotomurus palustris* (Müll.) SAURE & BRUMMER-KORVENKONTIO (1958), next to six bivalents, observed an additional, minute and apparently univalent element which in their opinion represents an "accessory chromosome" (=sex chromosome?).

In lateral views of primary oocyte metaphase of *Entomobrya lanuginosa* (Nic.) SAURE & BRUMMER-KORVENKONTIO (1958) observed a bivalent with clearly heterokinetic behaviour ("autoorientation") and considered it to be a sex element.

From drawings given by TUZET & MANIER (1956) of spermatocytes of *Orchesella villosa* (Geoffr.), *Entomobrya* cf. *nivalis* (L.) (also probably in *Sminthurus viridis* L.) the X element appears heteropycnotic at prophase of these species. A heteropycnotic element was found by NUNEZ (1962) also in early spermatocyte prophase of *Entomobrya pseudodecora* Rap.

In XO/XX systems the sex element undergoes prereduction.

#### IV. Nature of the centromere

Observations on the nature of the centromere in Collembola are completely lacking. The minute size of the chromosomes and, consequently, the lack of any visible structures, make direct observation extremely difficult. For the same reason an experimental approach (e.g. X-ray treatment, etc.) is also likely to fail.

As has been demonstrated in an earlier paper (KIAUTA, 1969) paleontologically old orders of insects nearly without exception possess diffuse kinetochores. On the strength of this evidence the diffuse condition of the centromere could be tentatively expected in Collembola as well. The difficulty, however, lies in the fact, that according to recent and well grounded views (cf. SHAROV, 1966) Protura, Collembola, Diplura and Insecta (Thysanura and Pterygota) apparently represent four separate lines of evolution of hexapod Atelocerata which developed independently from Protosymphylla. All four groups should be regarded as independent classes; therefore our conclusions on the distribution of types in insects should not be applied to the apterygotan classes as well.

Against the diffuse nature of the centromere in Collembola is also the evidence on the relation between the chromosome number and the degree of advancement and specialisation. While in all insect orders possessing diffuse kinetochores and in which a large enough number of forms has been studied cytologically, phylogenetic advancement and specialisation are coupled with a general increase in chromosome number (KIAUTA, 1967; 1968), the situation in Collembola is exactly the reverse.

#### V. Taxonomic applications of cytological characters

In spite of the small number of forms studied, a few cytological characters came to light, which can be used to advantage in collembolan systematics:

- (1) Increase in advancement and specialisation is generally coupled with a decrease in chromosome number; the type number pattern is clearly distinct between Arthropleona and Symphypleona.
- (2) Most of the families are largely or entirely homogeneous as far as chromosome numbers are concerned, whereas *Neanuridae* are essentially heterogeneous from a cytological point of view.
- (3) The mode of sex determination is uniform at least in *Isotomidae* and *Entomobryidae*.
- (4) No numeric pattern is recognizable at the subfamily level in *Isotomidae*, *Entomobryidae* and *Sminthuridae*; it will probably turn up in *Neanuridae* when more material will have been examined.
- (5) In four of the five species of *Tomoceridae* (belonging to two genera) one extra large bivalent was observed for which a trivalent structure was suggested. If this is correct, the *Tomoceridae*, as far as the chromosome numbers are concerned, may well represent a link between *Isotomidae* ( $n = 7$ ) and *Entomobryidae* ( $n = 6$ ).
- (6) In view of the small material so far available, no patterns on the generic level were observed. The chromosome number of *Isotomina* is peculiar within the family, but this observation is based on a single species and is therefore inconclusive.
- (7) The single cytological distinction on the species level seems to be that in the chromosome number of *Proisotoma minuta* (Tullb.).
- (8) Numeric differences between individuals of the same species were found only in *Entomobrya pseudodecora* Rap.

The preparations and microphotographs of *Folsomia candida distincta*, *Tomocerus minor* and *Allacma fusca* were made by Mrs. H.C.M. ZWANENBEEK-SNELDER (Utrecht). For advice on the synonymy and system used in Table I thanks are due to Dr. W. ELLIS (Museum of Zoology, Amsterdam), whereas Mr. D. SMIT (Utrecht) took care of the illustrations.

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## HET KARYOTYPE VAN *CERCOPITHECUS HAMLYNI* (Pocock, 1907) (PRIMATES, CERCOPITHECIDAE)

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Sinds het door de methode van MOORHEAD et al. (1960) voor het kweken van leucocyten uit menselijk bloed, mogelijk is geworden om ook van zoogdieren op een vrij eenvoudige manier chromosoomportretten te verkrijgen, zijn vrijwel alle primaten cytologisch onderzocht.

Van het genus *Cercopithecus* (Linnaeus, 1758) waren, met uitzondering van *C. hamlyni* (Pocock, 1907), reeds in 1967 alle karyotypen bekend. *C. hamlyni*, de Uilekop-macaco, is een aap die verborgen leeft in de oerwouden van oostelijk Kongo, waardoor hij slechts zelden in dierentuinen voorkomt.

Onlangs werd ik door Artis in de gelegenheid gesteld bloed af te nemen van een ♂ van deze zeldzame soort. De leucocytenkweken hiervan leverden 49 goede metaphaseplaten op, waarvan 80% een chromosoomaantal van 64 bleek te hebben (Fig. 1).

Het karyotype telt dus 32 chromosoomparen. Deze paren zijn in drie vrij duidelijk omliggende groepen te verdelen:

- Een groep van 15 metacentrische of vrijwel metacentrische chromosomen, met armratio's van 1,0 tot 1,3.
- Een groep van 11 submetacentrische paren, waarvan de armratio's variëren van 2,0 tot 2,4.
- Een groep van 6 acrocentrische paren, waarbij met uitzondering van het laatste paar geen korte arm te onderscheiden is.

Van 9 op deze manier gerangschikte karyogrammen werden de lengten van de beide armen van ieder chromosoom gemeten, en met behulp van deze getallen werden van ieder chromosoompaar de gemiddelde relatieve armlengten uitgerekend, uitgedrukt in procenten van de gemiddelde totale haploïede chromosoomlengte. Deze waarden zijn uitgezet in een idiogram (Fig. 2).