

## A karyotype study on the pseudoscorpion families Geogarypidae, Garypinidae and Olpiidae (Arachnida: Pseudoscorpiones)

FRANTIŠEK ŠŤÁHLAVSKÝ<sup>1,2</sup>, JIŘÍ KRÁL<sup>2</sup>, MARK S. HARVEY<sup>3</sup> and CHARLES R. HADDAD<sup>4</sup>

<sup>1</sup>Department of Zoology, Faculty of Sciences, Charles University, Viničná 7, CZ-128 44 Prague 2, Czech Republic;  
e-mail: stahlf@natur.cuni.cz

<sup>2</sup>Laboratory of Arachnid Cytogenetics, Department of Genetics and Microbiology, Faculty of Sciences, Charles University, Viničná 5, CZ-128 44 Prague 2, Czech Republic; e-mail: spider@natur.cuni.cz

<sup>3</sup>Western Australian Museum, Locked Bag 49, Welshpool DC, Western Australia 6986, Australia;  
e-mail: mark.harvey@museum.wa.gov.au

<sup>4</sup>Department of Zoology and Entomology, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa;  
e-mail: haddadcr.sci@mail.uovs.ac.za

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**Abstract.** The karyotypes of pseudoscorpions of three families, Geogarypidae, Garypinidae and Olpiidae (Arachnida: Pseudoscorpiones), were studied for the first time. Three species of the genus *Geogarypus* from the family Geogarypidae and 10 species belonging to 8 genera from the family Olpiidae were studied. In the genus *Geogarypus* the diploid chromosome numbers of males range from 15 to 23. In the family Olpiidae the male chromosome numbers vary greatly, from 7 to 23. The male karyotype of single studied member of the family Garypinidae, *Garypinus dimidiatus*, is composed of 33 chromosomes. It is proposed that the karyotype evolution of the families Geogarypidae and Olpiidae was characterised by a substantial decrease of chromosome numbers. The diploid numbers of some olpiids are the lowest known 2n within pseudoscorpions and even one of the lowest within the class Arachnida. In spite of a considerable reduction of diploid numbers, all species studied possess a X0 sex chromosome system that is widespread and probably ancestral in pseudoscorpions. Moreover, X chromosomes retain conservative metacentric morphology in the majority of species. During the first meiotic division of males, a high number of chiasmata were observed in some species, up to five per bivalent in *Indolpium* sp. The transient stage between pachytene and diplotene is typically characterised by extensive decondensation of chromatin in males of geogarypids and in *Calocheiridium libanoticus*, and we interpret this as a diffuse stage. This is recorded in pseudoscorpions for the first time. The relationships between some species belonging to the family Olpiidae are discussed based on the data obtained.

### INTRODUCTION

The Class Arachnida forms a huge and archaic branch of the arthropods. Starting from the Middle Devonian almost all recent orders of arachnids have been found to be well differentiated (Selden et al., 1991). Despite the importance of arachnids, their cytogenetic systems are still poorly understood in comparison to other arthropods, especially insects. Moreover, most data were obtained from certain arachnid orders (spiders, mites, harvestmen, and scorpions) only, while other groups remain poorly understood or even untested (Král, 1994).

The order Pseudoscorpiones is the fourth largest order of arachnids and is divided into 25 families with more than 3200 described species (Harvey, 2002; Judson, 2005). From a karyological point of view, it remains a poorly investigated group. Karyotypes of only 25 species belonging to four families have been published to date. Basic information on gametogenesis and chromosomes of pseudoscorpions were presented by Sokolow (1926) in *Neobisium carcinoides* (Neobisiidae) and *Dendrochernes cyrneus* (Chernetidae), and by Boissin & Manier (1966) in *Hysterochelifer meridianus* (Cheliferidae). However, only recently has the cytogenetics of pseudoscorpions been studied more intensively. Basic descriptions of the

chromosome numbers and their morphology were performed in five representatives of the family Chernetidae (Šťáhlavský, 2000; Šťáhlavský et al., 2005), and two *Allochthonius* species (Chthoniidae) (Lee & Seo, 1995). Karyotype evolution was analysed for the first time by Troiano (1990, 1997), who studied six Italian species of the genus *Roncus* (Neobisiidae). A similar study was also conducted by Šťáhlavský & Král (2004), who investigated the karyotypes of nine European representatives of the family Chthoniidae. The data obtained were used to reconstruct the evolutionary relationships of the species studied. In both groups the evolution of karyotypes was characterised by a reduction of chromosome numbers. In the genus *Roncus* the main chromosome rearrangements are probably centric fusions (Troiano, 1997), while study of the genus *Chthonius* indicates operation of centric fusions, tandem fusions, and pericentric inversions in the evolution of the genus (Šťáhlavský & Král, 2004). In some lineages of Mediterranean chthoniids, multiple fusions led to the origin of conspicuous “macrochromosomes” (Šťáhlavský & Král, 2004).

From all of the above-mentioned studies it is obvious that sex chromosomes are well differentiated in pseudoscorpions. The most common type of sex chromosome determination is the system X0, which is probably ances-

tral in pseudoscorpions (Troiano, 1997; Šťáhlavský & Král, 2004). Another characteristic of pseudoscorpion karyotypes is the wide range in diploid chromosome numbers, from  $2n = 10$  in *Allochthonius buanensis* (Chthoniidae) (Lee & Seo, 1995) to  $2n = 74$  in *Lasiochernes cretonatus* (Chernetidae) (Šťáhlavský et al., 2005).

To supplement knowledge about the trends in karyotype evolution of pseudoscorpions, we concentrated the present study on three unstudied families, Geogarypidae, Garypinidae and Olpiidae. These groups are mainly tropical and subtropical in distribution but some species extend into temperate zones of the world. They belong to the microorder Mestommatina together with another five families characterised by the presence of long arolia (Harvey, 1992), which is a synapomorphy for this group even though considerable homoplasy occurs within the microorder. The Mestommatina is divided into two groups: the superfamily Garypoidea and the superfamily Olpioidea. The family Geogarypidae (superfamily Garypoidea) is a relatively small group that contains only 3 genera with 65 known species. The Garypinidae (superfamily Olpioidea) were recently separated from the Olpiidae by Judson (2005), who noted sufficient differences from many of the olpiids to warrant family-level recognition. The Garypinidae contains 20 genera and 74 species. The family Olpiidae (superfamily Olpioidea) contains 22 genera and 193 species (Harvey, 1991).

## MATERIAL AND METHODS

Pseudoscorpions were sifted from leaf litter or collected individually from under stones or the bark of trees. Unless otherwise indicated, specimens were collected, determined, and deposited in the first author's collection (Department of Zoology, Charles University, Prague). Material from Western Australia and some specimens from South Africa are deposited in the Western Australian Museum in Perth, Australia (WAM). The detailed collection data are presented below. Species are ordered in the text, tables and figures from the highest to the lowest number of chromosomes within the families.

### Geogarypidae

*Geogarypus* sp.: South Africa: KwaZulu-Natal, Ndumo Game Reserve (9♂, 4♀: 6.–10.ii.2005).

*Geogarypus nigrimanus* (E. Simon, 1879): Greece: Corfu – Agios Georgios (6♀, 1 male tritonymph: 8.iv.2004), Ágrafi (1♂: 8.v.2002), Antiniotisa lake (1♀: 16.iv.2004), Kassiope (3♀: 24.iv.2001), lake Corission (1♂: 23.iv.2001), Loutsas (2♂: 15.iv.2004), Paleokastrica (2♂, 1♀: 9.iv.2004).

*Geogarypus taylora* Harvey, 1986: Western Australia: John Forrest National Park, under bark of *Eucalyptus* sp. (4♂, 4♀, 1 tritonymph: 20.viii.2003), lgt. M. Štambergová and F. Šťáhlavský, det. M. Harvey, coll. WAM.

### Garypinidae

*Garypinus dimidiatus* (L. Koch, 1873): Greece: Corfu – Agios Georgios (2♂: 8.iv.2004), Ágrafi (1♂: 8.v.2002), Antiniotisa lake (1♂, 1♀: 16.iv.2004), Kassiope (2♂, 3♀: 24.iv.2001; 1♂, 1♀: 8.v.2002), lake Corission (2♀: 23.iv.2001), Loutsas (1♀: 15.iv.2004).

### Olpiidae

*Nanolpium* sp.: South Africa: KwaZulu-Natal: Ndumo Game Reserve (4♂: 6.–10.ii.2005), lgt. D. Kunz, det. M. Harvey, coll. WAM.

*Horus obscurus* (Tullgren, 1907): South Africa: Soetdoring Nature Reserve, leaf litter (1♂, 1♀: spring 2004), lgt. C. Haddad, det. M. Harvey, coll. WAM.

*Calocheirus atopus* (Chamberlin, 1930): Israel: Hatzeva (1♂: 12.iii.2001), lgt. S. Pekár; Sde Boker (3♂: 26.iv.2001), lgt. S. Pekár, (1♂, 1♀: 20.iv.2004), lgt. M. Řezáč.

*Austrohorus* sp. 1: Western Australia: Karijini National Park, (1♂, 1♀: 9.ix.2003), det. M. Harvey, coll. WAM.

*Austrohorus* sp. 2: Western Australia: Kalbarri National Park – the Loop, under stones (2♂: 2.ix.2003), lgt. M. Štambergová, det. M. Harvey, coll. WAM.

*Mimmiza babylonica* Beier, 1929: Israel: Sde Boker (4♂, 1♀: 30.iii.–26.iv.2001), lgt. S. Pekár, det. V. Mahnert.

*Calocheiridius libanoticus* Beier, 1955: Turkey – Huzurkent (8 km from Tarsus) (1♂: 20.iv.2002), lgt. J. Skuhrovec, det. V. Mahnert.

*Olpium turcicum* Beier, 1949: Israel: Sde Boker, Haluquim Ridge (3♂: 1.–10.xii.2004), lgt. J. Král, det. V. Mahnert.

*Olpium pallipes* (Lucas, 1849): Greece: Corfu – lake Corission (1♀: 23.iv.2001; 1♂, 5♀: 7.v.2002; 2♂, 6♀: 13.iv.2004); Portugal: Algarve, Aljezur, Praia da Bordeira near Lagos, cliffs and sand dunes (2♀: 12.iv.2005), lgt. M. Řezáč, det. V. Mahnert.

*Indolpium* sp.: Western Australia: Kalbarri National Park, the Loop, under stones (1♂: 2.ix.2003), lgt. M. Štambergová, det. M. Harvey, coll. WAM.

## Methods

Chromosome preparations were made following the technique described in detail by Šťáhlavský & Král (2004). Briefly, the gonads or the whole content of the opisthosoma were hypotonised in 0.075 M KCl for 15 min in geogarypids, and for 10 min in olpiids and garypinids. The tissues were then fixed in a glacial acetic acid : methanol (1 : 3) solution for at least 20 min. After fixation a suspension was prepared in a drop of 60% acetic acid on a microscope slide. The preparation was then displaced on a warm histological plate (temperature 40–45°C); the drop of suspension was moved on the surface of the slide by tungsten needle until the drop had evaporated. Chromosome preparations were stained with 5% Giemsa solution in Sörensen phosphate buffer (pH = 6.8) for 30 min. in geogarypids, and for 20 min in olpiids and garypinids.

To obtain data on chromosome morphology, ten plates of a suitable stage (mitotic metaphase or metaphase II) were measured and evaluated. In *Geogarypus* sp., the centromeric area was marked by a prominent knob during diplotene, which enabled us to identify the centromere and also use these plates for the evaluation of chromosome morphology. Relative chromosome lengths (Tables 1, 2) were calculated as a percentage of the total chromosome length of the haploid set (TCL), including the sex chromosome. Chromosome morphology was classified according to Levan et al. (1964). In species where a sufficient number of diplotene or diakinesis was obtained, the mean chiasma frequency was calculated per cell and per bivalent (Table 3). The mean chiasma frequency per bivalent was calculated as the ratio between the mean chiasma frequency per cell and number of bivalents. In olpiids with  $2n = 7$ , mean values of relative chromosome lengths (% TCL) as well as arm ratio of corresponding autosome pairs and X chromosome was compared by nonparametric Mann-Whitney U Test (Table 4).

TABLE 1. Genus *Geogarypus*. Chromosome relative lengths (% TCL) and centromeric indexes (AR) of species studied (mit – mitotic metaphase, met II – metaphase II, dip – diplotene).

Pair No.	<i>Geogarypus</i> sp.		<i>G. nigrimanus</i>				<i>G. taylori</i>			
	male – dip		male – met II		female – mit		male – met II		female – mit	
	% TCL	AR	% TCL	AR	% TCL	AR	% TCL	AR	% TCL	AR
1	10.60	1.30	13.44	2.66	14.95	2.56	17.44	1.17	22.25	1.15
2	9.74	1.65	11.15	1.24	11.87	1.11	13.06	1.15	15.08	1.16
3	9.17	1.27	10.59	1.49	11.14	1.25	12.53	1.27	11.84	1.18
4	7.94	1.26	9.93	1.14	10.21	1.18	12.14	1.13	11.00	1.17
5	7.59	1.41	9.08	1.31	9.33	1.25	11.58	1.28	10.49	1.12
6	7.26	2.39	7.95	1.75	7.30	1.45	11.35	1.48	10.26	1.33
7	7.21	1.26	7.58	1.27	7.08	3.74	9.84	1.21	9.62	1.17
8	6.42	1.37	6.87	3.07	6.37	1.32			9.46	1.45
9	6.40	1.73	6.76	1.55	5.91	3.83				
10	5.84	1.17								
11	5.82	1.64								
X	15.90	1.32	16.82	1.17	16.24	1.26	12.06	1.25		

## RESULTS

### Geogarypidae

#### *Geogarypus* sp.

The male chromosome complement is composed of 23 chromosomes, namely nine pairs of metacentric and two pairs of submetacentric autosomes, and metacentric X chromosome. Autosomes gradually decrease in size; their relative lengths range from 10.06% to 5.82% of TCL in male diplotene (Table 1). The X chromosome is the longest element of the karyotype (15.90% of TCL).

Only some meiotic stages were observed. In pachytene, the X chromosome is characterised by prominent negative heteropycnosis (Fig. 1A). The transient stage between pachytene and diplotene is marked by highly despiralised chromatin of chromosomes, with the exception of the centromere areas as well the X chromosome (Fig. 1B). In contrast to the transient stage, only the large proximal part of the short arm of X remains positively heteropycnotic during diplotene (Fig. 1C). This heteropycnotic segment forms up to 53.89% of the arm (mean  $\pm$  SD = 49.08%  $\pm$  4.8, N = 5). During this stage, the centromeric regions still formed prominent knobs; two to four ring bivalents were observed. The mean chiasma frequency is 14.00 per cell or 1.27 per bivalent, respectively (Table 3).

#### *Geogarypus nigrimanus*

The male karyotype comprises 19 chromosomes; the sex chromosome system is X0 (Fig. 2A). Male metaphase II shows 6 metacentric, 2 submetacentric and 1 subtelocentric autosomes. The length of the chromosome pairs gradually decreases in size from 13.44% to 6.76% of TCL (Table 1). In the mitotic metaphase of females, we found slight differences in the distribution of chromosome types. It contains only 1 submetacentric but 2 subtelocentric pairs of chromosomes. This difference is probably caused by different spiralisation of some chromosome pairs in the mitotic metaphase. The metacentric chromosome X is the largest chromosome in the karyotype, and thus, it can also be easily recognised in the mitotic meta-

phase of females (Fig. 2C). It forms 16.82% of TCL in metaphase II of males and 16.24% of TCL in female mitotic metaphase, respectively (Table 1).

We succeeded to investigate nearly the entire course of meiosis in this species. During the beginning of prophase I (leptotene and zygotene), the sex chromosome exhibits conspicuous positive heteropycnosis (Fig. 1D). In contrast to previous species, all chromosomes are isopycnotic in the pachytene (Fig. 1E). The transient stage between pachytene and diplotene is characterised by highly despiralised chromatin, with exception of the centromere areas as well as part of the X chromosome. The centromere areas are marked by a prominent knob and the proximal part of one X chromosome arm displays positive heteropycnosis (Fig. 1F). During diplotene and diakinesis, all chromosomes are isopycnotic. From metaphase II, the X chromosome starts to be positively heteropycnotic once again (Fig. 1H). This period of heteropycnosis culminates during following anaphase II (Fig. 1I).

Males of *G. nigrimanus* exhibit a higher chiasma frequency than the previous species during late prophase I. Only one bivalent has a single chiasma whereas all other bivalents usually possess two or even three chiasmata (Fig. 1G). The mean chiasma frequency is 16.87 per cell or 1.87 per bivalent, respectively (Table 3).

#### *Geogarypus taylori*

The diploid chromosome number of males is 15 and all chromosomes are metacentric (Fig. 2B). The relative lengths of the autosomes range from 17.44% to 9.84% of TCL in male metaphase II (Table 1). The metacentric X chromosome forms 12.06% of TCL. During this stage, the X chromosome is extremely superspiralised and thus exhibits prominent positive heteropycnosis. The mitotic diploid complement of the female contains 16 chromosomes; all chromosomes are metacentric, as in the male (Fig. 2D). In both sexes the first autosome pair is distinctly longer than all other chromosomes (Table 1). The

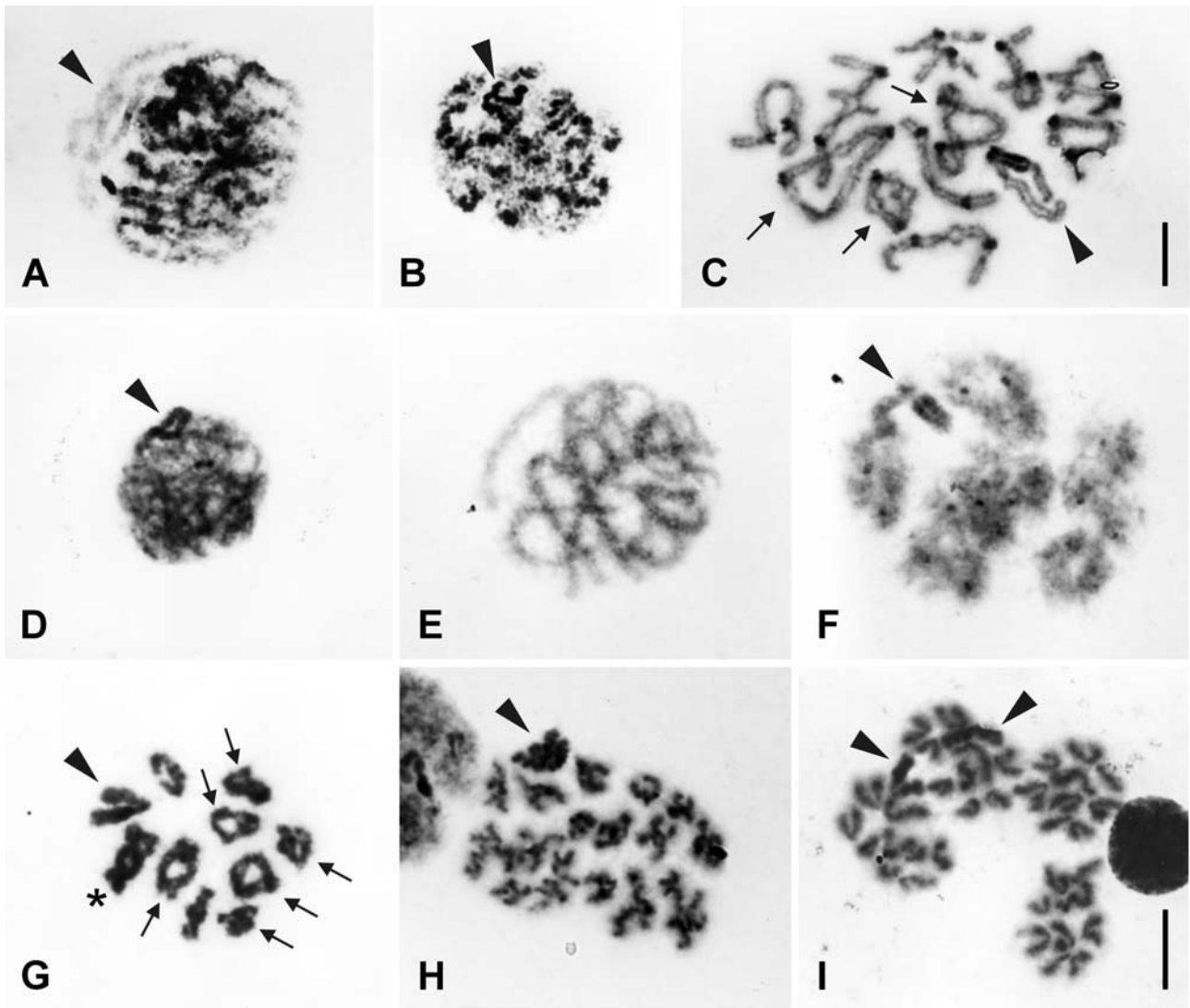


Fig. 1. Course of male meiosis in the genus *Geogarypus*. A – C *Geogarypus* sp. (South Africa). A – pachytene: sex chromosome exhibits negative heteropycnosis; B – diffuse stage: X chromosome is positively heteropycnotic; C – diplotene: part of sex chromosome displays positive heteropycnosis, three bivalents have two chiasmata. Scale bar = 10  $\mu$ m (valid for A–C). D – I *Geogarypus nigrimanus*. D – zygotene: sex chromosome exhibits positive heteropycnosis; E – pachytene: all chromosomes are isopycnotic; F – diffuse stage: part of the sex chromosome is positively heteropycnotic; G – diakinesis: all chromosomes are isopycnotic, one bivalent exhibits three chiasmata (asterisk); H – metaphase II: sex chromosome displays positive heteropycnosis; I – anaphase II: sex chromosome differs by positive heteropycnosis. Scale bar = 10  $\mu$ m (valid for D–I). Arrowheads indicate sex chromosomes; arrows show bivalents with two chiasmata.

X chromosomes can therefore not be recognised based on size or morphology in the mitoses of females (Fig. 2D).

Despite the fragmentary data obtained, the course of meiotic division seems to be similar to *G. nigrimanus*. During leptotene and zygotene the sex chromosome exhibits positive heteropycnosis. On the other hand, all chromosomes are isopycnotic during pachytene, including the X chromosome. In metaphase II, the sex chromosome is newly positively heteropycnotic. Heteropycnosis during metaphase II is more prominent than in the previous species.

### Garypinidae

#### *Garypinus dimidiatus*

The diploid chromosome number is 33 in males (Fig. 3A) and 34 in females (Fig. 3B). The male karyotype

comprises 8 metacentric and 8 submetacentric pairs of autosomes, and one large submetacentric X chromosome (it forms 10.06% of TCL). The relative lengths of the autosomes decrease gradually from 8.23% in the first pair to 4.47% of TCL in pair No. 14. The last two autosome pairs are much shorter than the preceding ones (they form 2.90% and 2.59% of TCL, respectively) (Table 2). Considering meiosis, we only observed metaphase II of males. During this stage, the X chromosome displays negative heteropycnosis (Fig. 6J).

### Olpiidae

#### *Nanolpium* sp.

The male diploid complement contains 23 chromosomes (Fig. 3C). The male karyotype consists of three pairs of metacentric and eight pairs of acrocentric auto-

TABLE 2. Families Garypinidae and Olpiidae. Chromosome relative lengths (% TCL) and centromeric indexes (AR) of species studied (mit – mitotic metaphase, met II – metaphase II).

Pair No.	<i>Garypinus dimidiatus</i>				<i>Nanolpium</i> sp.		<i>Horus obscurus</i>		<i>Calocheirus atopos</i>		<i>Minniza babylonica</i>	
	male – met II		female – mit		male – met II		female – mit		male – mit		female – mit	
	% TCL	AR	% TCL	AR	% TCL	AR	% TCL	AR	% TCL	AR	% TCL	AR
1	8.23	1.82	8.34	2.22	12.19	1.45	12.68	1.12	11.26	1.50	20.13	1.13
2	7.43	2.48	7.77	1.86	10.97	1.24	11.29	1.64	10.15	1.25	16.13	1.53
3	7.22	2.16	7.69	2.60	9.83	1.28	9.55	1.43	9.42	1.17	16.53	2.17
4	6.83	2.97	7.24	2.79	8.75	–	8.35	1.13	9.20	1.27	14.24	1.41
5	6.62	2.40	7.04	1.65	7.83	–	8.28	1.15	8.87	1.49	13.74	1.27
6	6.31	1.67	6.20	1.90	7.38	–	7.45	1.59	8.30	1.38	10.43	1.19
7	5.92	1.62	5.89	1.68	7.09	–	7.20	1.24	7.95	1.29	9.20	1.20
8	5.78	1.16	5.87	1.34	6.76	–	5.72	1.82	7.72	2.38		
9	5.46	1.70	5.32	1.13	6.35	–	5.63	1.23	6.98	2.45		
10	5.35	1.72	5.07	1.30	5.98	–	5.03	5.33	6.80	3.35		
11	5.11	1.66	4.98	1.66	5.54	–						
12	4.97	1.89	4.78	2.24								
13	4.74	1.56	4.71	1.28								
14	4.47	1.63	4.07	1.97								
15	2.90	2.20	3.17	1.53								
16	2.59	1.51	3.01	1.98								
X	10.06	1.78	8.93	1.71	11.33	1.71	18.82	1.18	13.36	1.52		
Pair No.	<i>Calocheiridius libanoticus</i>		<i>Olpium turcius</i>		<i>Olpium pallipes</i>				Portugal		<i>Indolpium</i> sp.	
	male – mit		male – mit		Greece – Corfu		female – mit		female – mit		male – met II	
	% TCL	AR	% TCL	AR	% TCL	AR	% TCL	AR	% TCL	AR	% TCL	AR
1	19.90	7.83	35.57	1.10	35.97	1.36	39.09	1.58	40.82	1.38	36.64	1.27
2	18.00	5.32	28.97	1.09	32.97	1.13	31.07	1.17	31.97	1.25	32.05	1.12
3	16.50	8.24	13.15	–	10.88	1.09	9.61	1.18	8.47	1.39	9.05	3.38
4	13.61	9.09										
5	9.40	1.34										
X	22.58	1.13	22.23	1.10	20.19	–	20.33	–	18.73	1.15	22.26	1.16

somes, and one large submetacentric X chromosome. More accurately, the X chromosome is metacentric in some cases (mean arm ratio  $\pm$  SD =  $1.71 \pm 0.16$ ) (Table 2). Autosomes gradually decrease in size from 12.19% to 5.54% of TCL. In meiosis, we found an enhanced chiasma frequency: three to eight of the bivalents possess two chiasmata, and one bivalent contains even three chiasmata in four cases. Mean chiasma frequency was counted as 14.40 per cell (1.31 per bivalent) (Table 3). In contrast to the other species analysed, chiasmata of ring bivalents are located more proximally, being interstitial or even pericentromeric (Fig. 6H).

#### *Horus obscurus*

Data on the male karyotype are based on plates of prophase I only; diplotene consists of 10 autosome bivalents and a metacentric univalent X (Fig. 6F). The female mitotic metaphase comprises 22 chromosomes (Fig. 4A). Almost all autosomes of the female are metacentric, with the exception of submetacentric pair No. 8 and subtelocentric pair No. 10. Autosomes gradually decrease in size from 12.68% to 5.03% of TCL. The female karyotype

contains a pair of large metacentric X chromosomes that form 18.82% of TCL (Table 2).

At the male diplotene, the whole sex chromosome exhibits positive heteropycnosis. During this stage, quite a high number of chiasmata per cell was observed (Fig. 6F), ranging from 11 to 15. Mean chiasma frequency is 12.69 per cell (1.27 per bivalent) (Table 3). In one case, one bivalent exhibits even three chiasmata (Fig. 6F).

#### *Calocheirus atopos*

The male mitotic metaphase includes 21 chromosomes (Fig. 4B). Autosome pairs gradually decrease in size from 11.26% to 6.80% of TCL. Metacentric chromosomes predominate in this species; seven autosome pairs are metacentric whereas only two short pairs are submetacentric (No. 8 and 9); the shortest pair is subtelocentric (Table 2). The metacentric X chromosome (arm ratio 1.52) is the largest chromosome in the karyotype. It forms 13.36% of TCL (Table 2) and is thus clearly discernible in the mitotic metaphases too. Concerning meiosis, we obtained only pachytene (Fig. 6B) and diplotene (Fig. 6G) of males. In both stages the X chromosome exhibits promi-

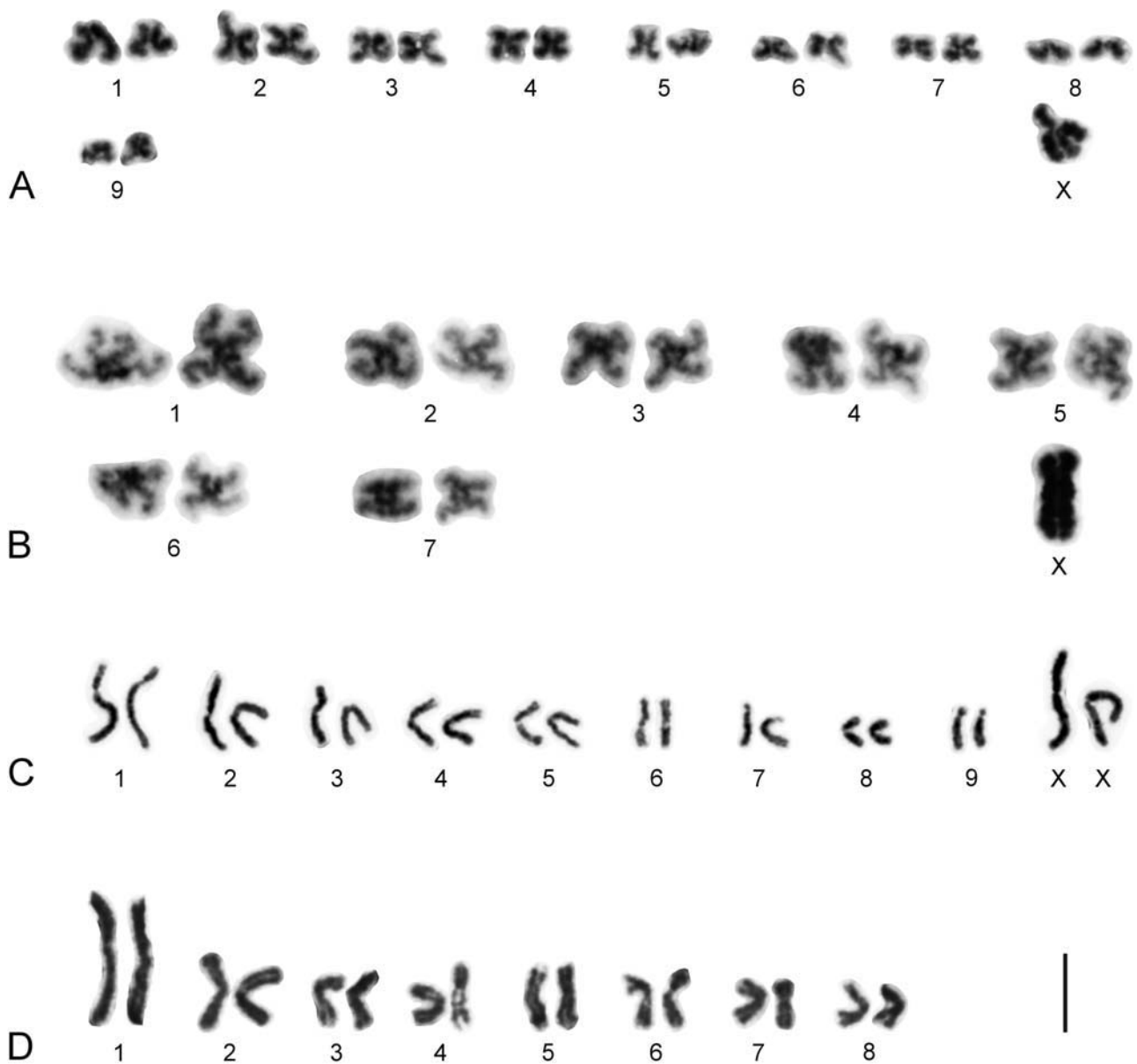


Fig. 2. A – D Karyotypes of the genus *Geogarypus*. A – male of *G. nigrimanus*,  $2n = 19$ , X0 (assembled from 2 sister metaphase II plates); B – male of *G. taylori*,  $2n = 15$ , X0 (assembled from 2 sister metaphase II plates); C – female of *G. nigrimanus*,  $2n = 20$ , XX (mitotic metaphase); D – female of *G. taylori*,  $2n = 16$ , XX (mitotic metaphase). Scale bar = 10  $\mu$ m.

ment positive heteropycnosis. In the diplotene, ten bivalents and a univalent X are visible. The majority of bivalents include one chiasma; two chiasmata were observed

in 2–5 bivalents. Mean chiasma frequency is 12.67 per cell (Table 3).

TABLE 3. Mean chiasma frequency in selected species of the families Geogarypidae and Olpiidae.

Species	2n	No. of analysed cells	Range of chiasma frequency per cell	Mean chiasma frequency	
				per cell	per bivalent
<i>Geogarypus</i> sp.	23	5	13–15	14.00	1.27
<i>Geogarypus nigrimanus</i>	19	83	16–20	16.87	1.87
<i>Nanolpium</i> sp.	23	53	15–19	14.40	1.31
<i>Horus obscurus</i>	21	19	11–15	12.69	1.27
<i>Calocheirus atopos</i>	21	18	11–14	12.67	1.27
<i>Austrohorus</i> sp. 1	19	15	9–10	9.53	1.06
<i>Austrohorus</i> sp. 2	19	26	9–11	9.50	1.06

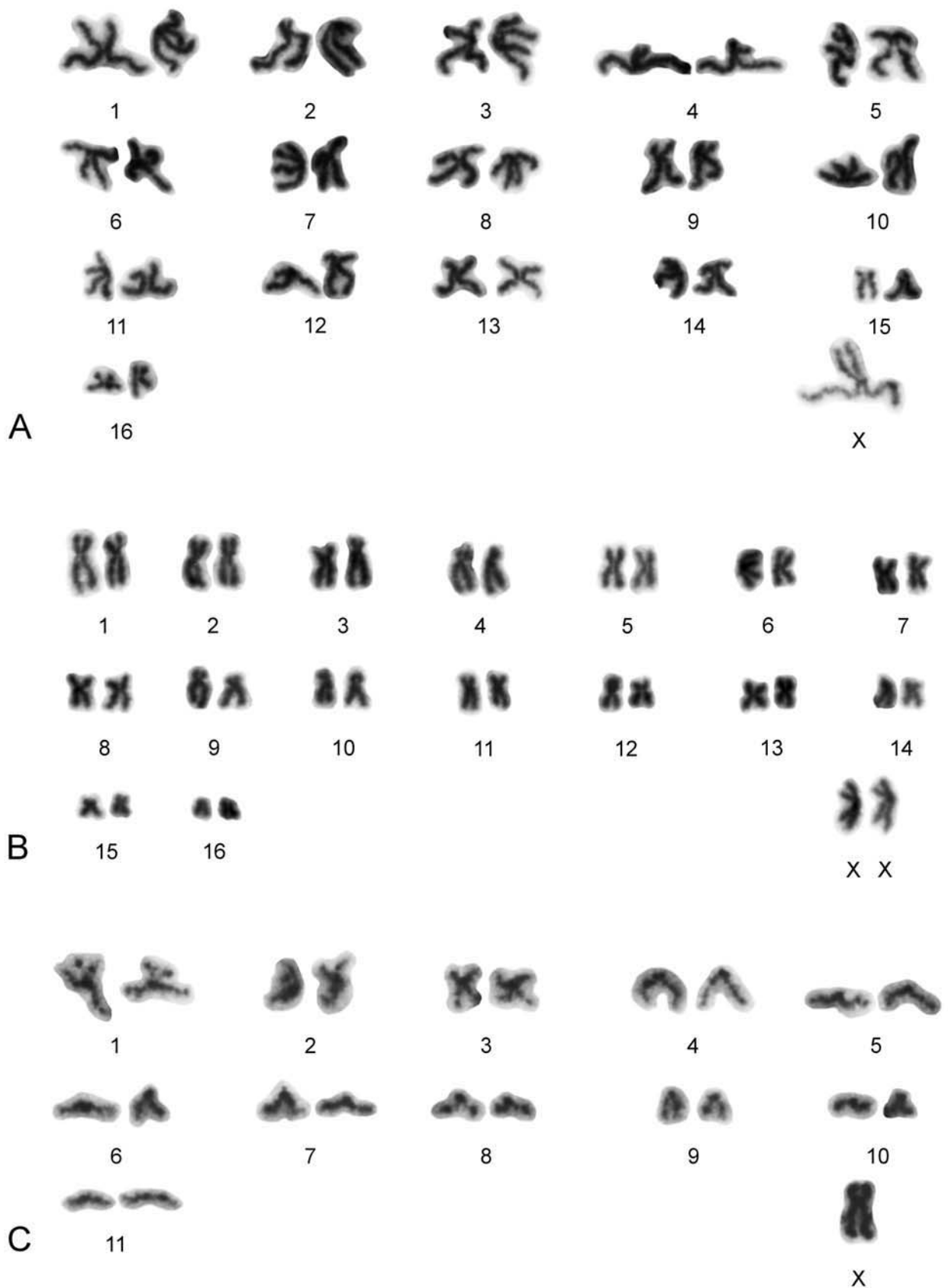


Fig. 3. A – C Karyotypes of garypinids and olpiids. A – male of *Garypinus dimidiatus*,  $2n = 33$ , XO (assembled from 2 sister metaphase II plates); B – female of *G. dimidiatus*,  $2n = 34$ , XX (mitotic metaphase); C – male of *Nanolpium* sp.,  $2n = 23$ , XO (assembled from 2 sister metaphase II plates). Scale bar = 10  $\mu$ m.

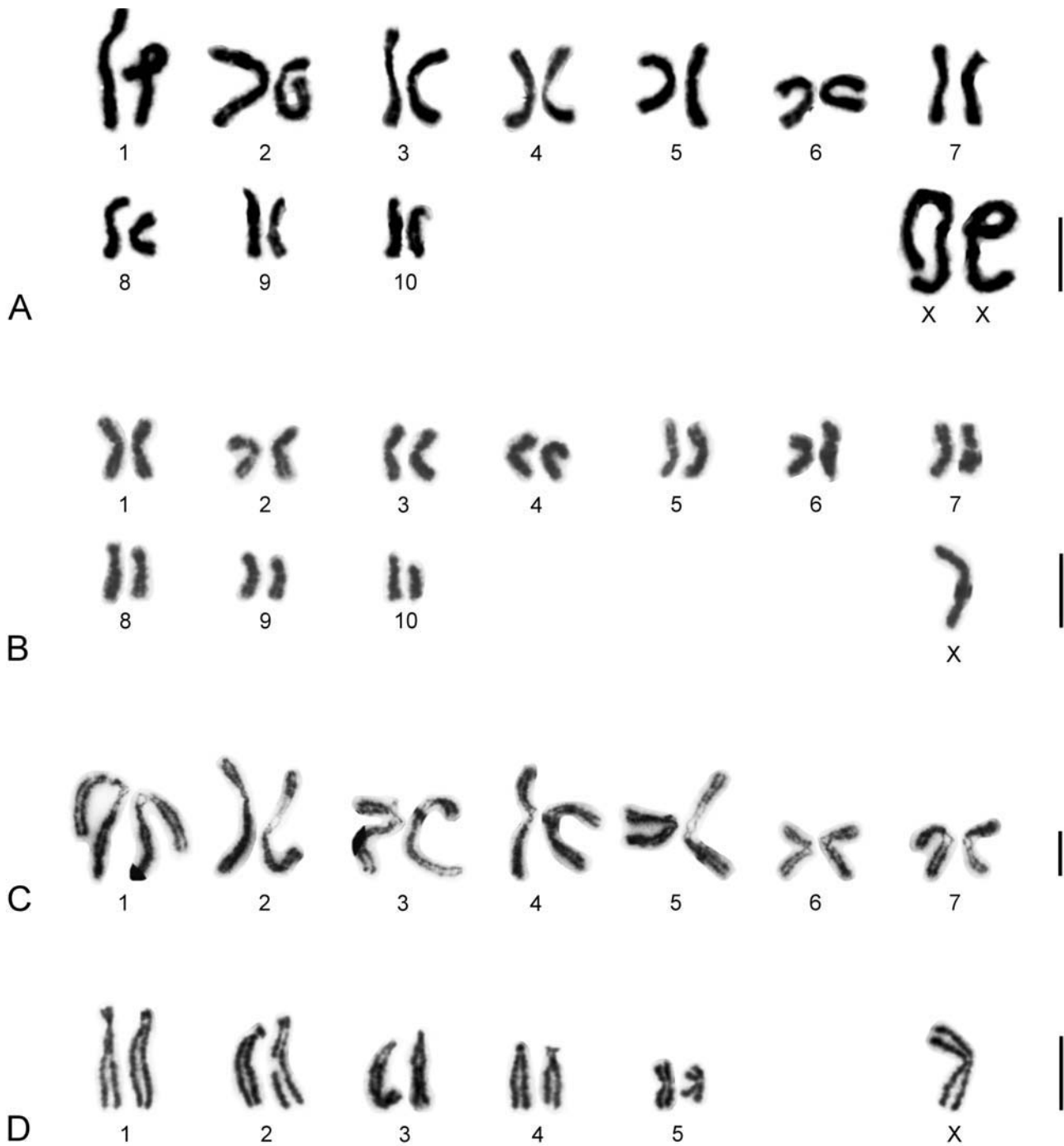


Fig. 4. A – D Karyotypes of oliiids constructed from mitotic metaphases. A – female of *Horus obscurus*,  $2n = 22$ , XX; B – male of *Calocheirus atopus*,  $2n = 21$ , XO; C – female of *Minniza babylonica*,  $2n = 14$ , XX; D – male of *Calocheiridius libanoticus*,  $2n = 11$ , XO. Scale bars = 10  $\mu\text{m}$ .

#### *Austrohorus* sp. 1 and *Austrohorus* sp. 2

In these two species from Western Australia we only obtained the diplotene stage. Diplotene plates of both species contain nine bivalents and one short univalent that represents the X chromosome (Figs 6D, 6E). In diplotene, the majority of bivalents bear one chiasma only. However, some plates contained one (*Austrohorus* sp. 1) or two bivalents (*Austrohorus* sp. 2) with two chiasmata. Chiasma frequency is nearly the same in both species.

Mean chiasma frequency is 9.53 per cell in *Austrohorus* sp. 1 and 9.50 in *Austrohorus* sp. 2 (Table 3).

#### *Minniza babylonica*

Chromosome plates were only obtained in females; mitotic metaphase consists of 14 chromosomes (Fig. 4C). Metacentric chromosomes predominate in the karyotype, and only the second pair of autosomes is submetacentric. Chromosomes gradually decrease in size from 20.13% to



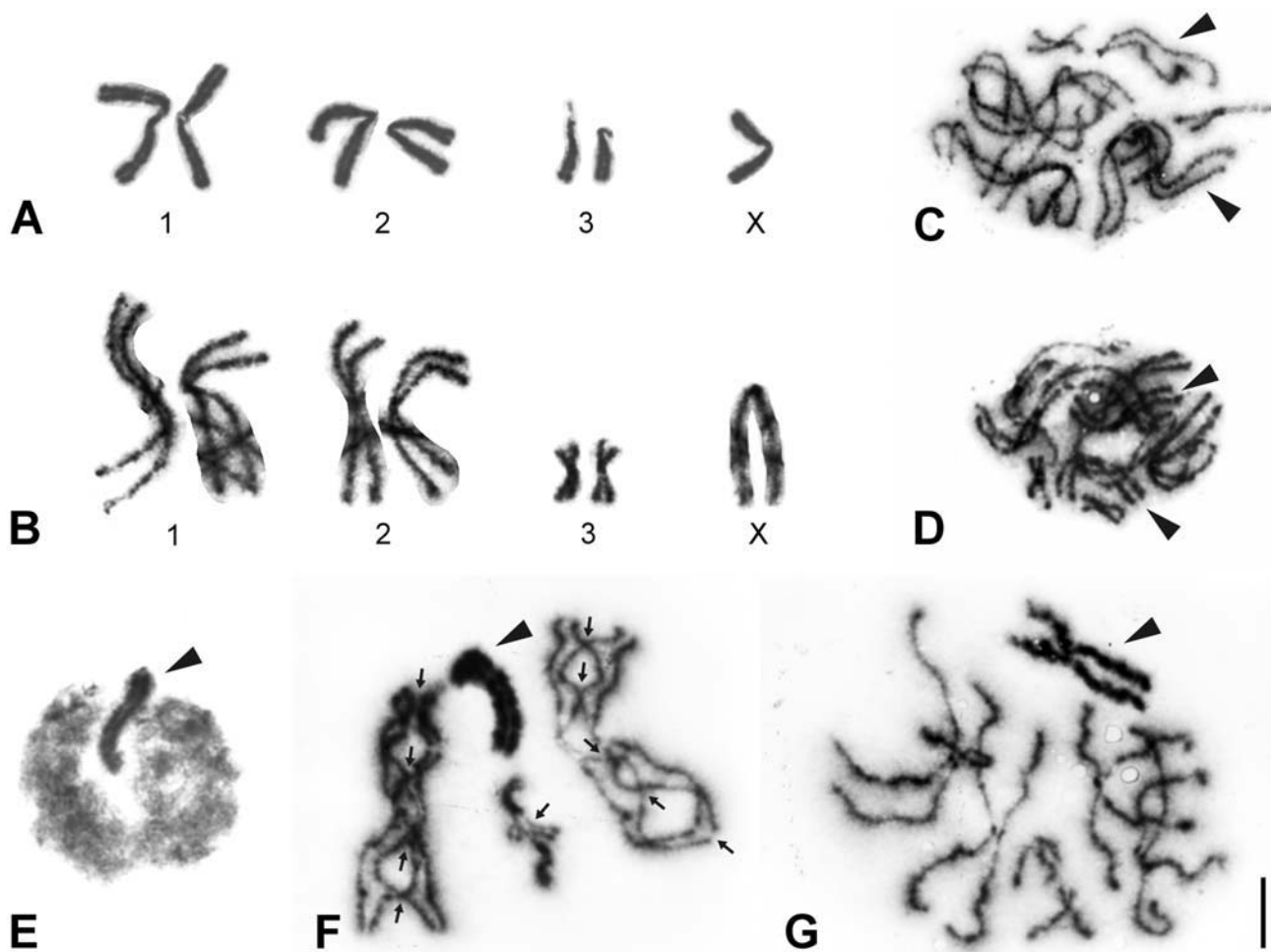


Fig. 5. A – B Karyotypes of oliptids with the lowest number of chromosomes; constructed from mitotic metaphases. A – male of *Olpium turcicus*,  $2n = 7, X0$ ; B – male of *O. pallipes* (Corfu),  $2n = 7, X0$ ; C – female mitotic metaphase of *O. pallipes* (Corfu): note acrocentric X chromosomes; D – female mitotic metaphase of *O. pallipes* (Portugal): metacentric morphology of all chromosomes indicates that X chromosomes are metacentric; E – G male meiosis of *Indolpium* sp.,  $2n = 7, X0$ . E – leptotene; F – diplotene: numerous chiasmata are indicated by arrows; G – early anaphase I. Arrowheads show sex chromosomes. Scale bar = 10  $\mu\text{m}$ .

TABLE 4. Oliptids with  $2n = 7$ . Comparison of karyometric data of particular chromosome pairs by Mann-Whitney U Test. % TCL – mean value of chromosome relative length.

Compared species	Pair No.	% TCL			Arm ratio		
		U	Z	p-level	U	Z	p-level
<i>Olpium turcicus</i> × <i>O. pallipes</i>	1	193	-0.98	0.33	35	-4.75	0.00
<i>Olpium turcicus</i> × <i>O. pallipes</i>	2	58	-4.20	0.00	184	-1.19	0.23
<i>Olpium turcicus</i> × <i>O. pallipes</i>	3	53	4.32	0.00	0	5.59	0.00
<i>Olpium turcicus</i> × <i>O. pallipes</i>	X	105	3.08	0.00	0	-5.59	0.00
<i>Olpium turcicus</i> × <i>Indolpium</i> sp.	1	102	-1.70	0.09	58	-3.08	0.00
<i>Olpium turcicus</i> × <i>Indolpium</i> sp.	2	27	-4.05	0.00	126	-0.94	0.35
<i>Olpium turcicus</i> × <i>Indolpium</i> sp.	3	0	4.90	0.00	11	4.55	0.00
<i>Olpium turcicus</i> × <i>Indolpium</i> sp.	X	141	-0.47	0.64	64	-2.89	0.00
<i>Olpium pallipes</i> × <i>Indolpium</i> sp.	1	82	-1.10	0.27	75	1.40	0.16
<i>Olpium pallipes</i> × <i>Indolpium</i> sp.	2	97	0.47	0.64	106	-0.08	0.93
<i>Olpium pallipes</i> × <i>Indolpium</i> sp.	3	15	3.94	0.00	0	-4.57	0.00
<i>Olpium pallipes</i> × <i>Indolpium</i> sp.	X	47	-2.58	0.01	0	4.57	0.00

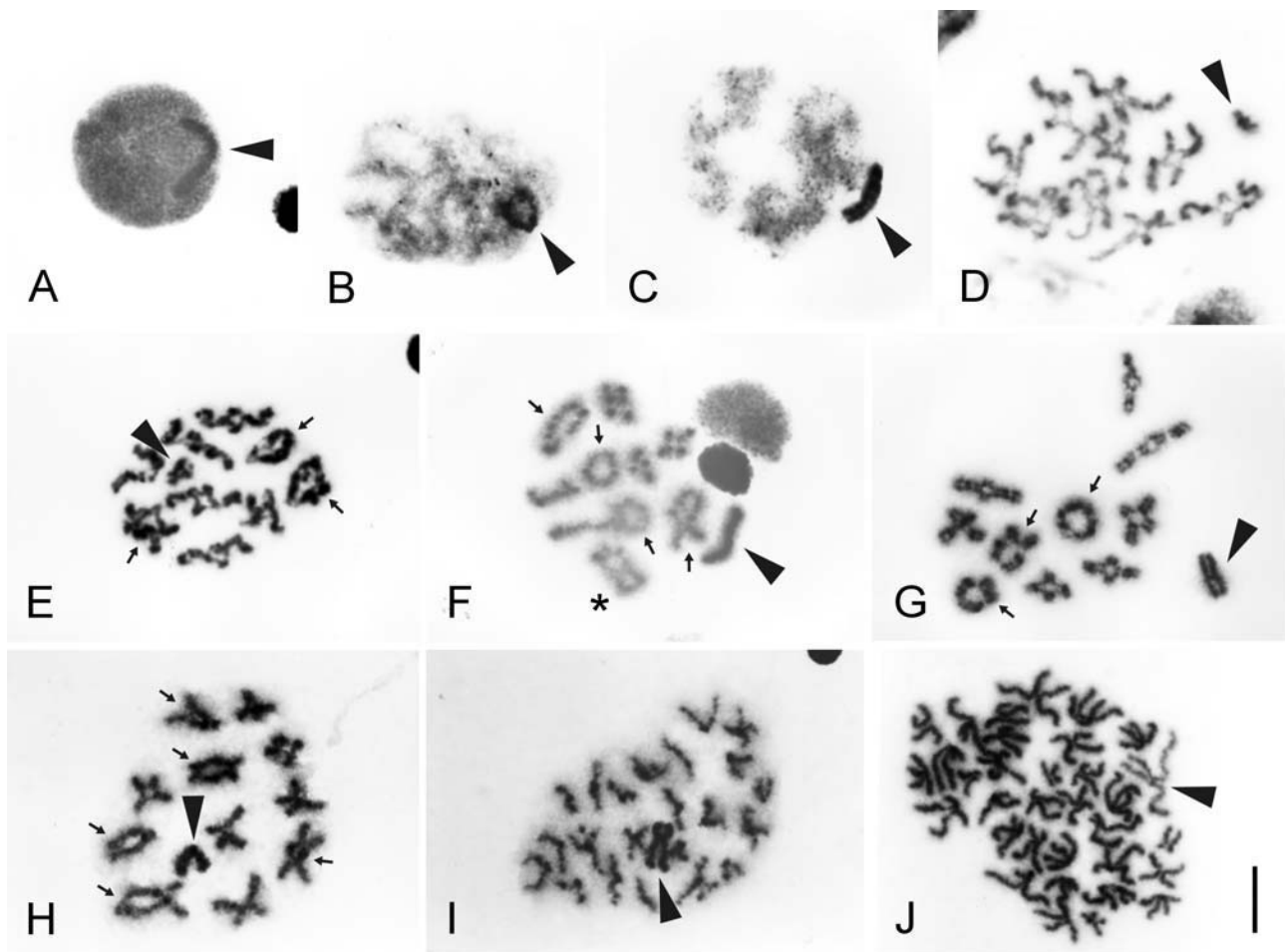


Fig. 6. A – J Course of meiosis in oliptids and garypinids. A – *Horus obscurus*, interphase before meiotic division: the X chromosome is positively heteropycnotic; B – *Calocheirus atopos*, pachytene: sex chromosome displays positive heteropycnotic; C – *Calocheiridius libanoticus*, diffuse stage: X chromosome possesses positive heteropycnotic; D – *Austrohorus* sp. 1, diplotene; E – *Austrohorus* sp. 2, diplotene; F – *Horus obscurus*, diplotene: note positive heteropycnotic of X chromosome, one bivalent exhibits three chiasmata (asterisk); G – *Calocheirus atopos*, diplotene: sex chromosome is positively heteropycnotic; H – *Nanolpium* sp., diplotene: note positive heteropycnotic of X chromosome; I – *Nanolpium* sp., metaphase II: X chromosome displays positive heteropycnotic; J – *Garypinus dimidiatus*, metaphase II: X chromosome exhibits negative heteropycnotic. Arrowheads indicate sex chromosomes; arrows show bivalents with two chiasmata. Scale bar = 10  $\mu$ m.

9.20% (Table 2). We were not able to distinguish sex chromosomes, neither according to size nor morphology.

#### *Calocheiridius libanoticus*

The male diploid chromosome number is 11 (Fig. 4D). In contrast to the majority of oliptid species studied, acrocentric chromosomes predominate in the karyotype of *C. libanoticus*. The autosome complement consists of three pairs of acrocentric, one pair of subtelocentric (No. 2) and one pair of metacentric chromosomes (No. 5). Autosome pairs gradually decrease in size from 19.90% to 9.40% of TCL (Table 2). The metacentric chromosome X is the largest chromosome of the haploid set (22.58% of TCL). Among rare figures of male prophase I, we observed plates characterised by extensive decondensation of autosome chromatin. However, chromosome X is superspiralised and displays positive heteropycnotic in this stage (Fig. 6C).

#### *Olpium turcicum*

The male mitotic metaphase contains 7 chromosomes only (Fig. 5A). Chromosome pairs No. 1 and 2 are large metacentrics; they form nearly two thirds (64.53%) of TCL. In contrast to this, pair No. 3 is acrocentric and only occupies 13.15% of TCL (Table 2). Chromosome pair No. 1 bears a conspicuous secondary constriction at the subterminal part of the long arm. This species possesses a X0 sex chromosome system with a metacentric X chromosome; its relative length is 22.23% of TCL.

#### *Olpium pallipes*

The male mitotic metaphase contains 7 chromosomes (Fig. 5B). The first two autosome pairs are formed by similar metacentric chromosomes of enormous size; the length of the hypotonised autosome No. 1 is approximately 29  $\mu$ m. The first and second autosome pairs occupy 35.97% and 32.97% of TCL, respectively. The third autosome pair is formed by shorter metacentrics; it

forms 10.88% of TCL (length of the hypotonised chromosome No. 3 is approximately 9  $\mu\text{m}$ ) (Table 2). This species displays a X0 sex chromosome system. In contrast to other olpiids analysed, the X chromosome is acrocentric in the Corfu population (Figs 5B, 5C). In males, it forms 20.19% of TCL (Table 2); its size is around 16  $\mu\text{m}$  in hypotonised mitotic metaphases.

In this species, we also had the chance to obtain mitotic plates from two females of a population from Portugal. Interestingly, the mitotic metaphases are composed of metacentric chromosomes only (Fig. 5D). This finding indicates that the X chromosome is metacentric. The autosome pairs possess similar length and morphology to the population from Corfu (Table 2).

#### *Indolpium* sp.

The male karyotype of this species from Western Australia is only composed of 7 chromosomes (Fig. 5G), as in *Olpium turcicum* and *O. pallipes*. The autosome complement is very similar to *O. pallipes* from South Europe. The first two autosome pairs are formed by large metacentric chromosomes; each of them forms more than a third of TCL. The last autosome pair is represented by shorter subtelocentrics that form 9.5% of TCL (Table 2). *Indolpium* sp. exhibits a large metacentric sex chromosome.

Positive heteropycnosis was observed in the X chromosome from the beginning of the first meiotic division (Fig. 5E) up to the early anaphase I (Fig. 5G). During diplotene, a high number of chiasmata per bivalent was observed (Fig. 5F). Large bivalents formed by metacentric autosomes possess four to five chiasmata, with two or even three on each chromosome arm. The shorter subtelocentric autosome pair exhibits one or two chiasmata (in the latter case it forms a ring bivalent).

## DISCUSSION

Pseudoscorpions are poorly characterised chromosomally. In the present study, we described the karyotypes of the microorder Mestommatina, namely representatives of the families Geogarypidae, Garypinidae and Olpiidae, for the first time. In the sole member of the family Garypinidae studied, the diploid number was quite high (male  $2n = 33$ ). The families Geogarypidae and Olpiidae are typically characterised by low numbers of chromosomes. Based on results previously obtained in pseudoscorpions (Troiano, 1990, 1997; Štáhlavský, 2000; Štáhlavský et al., 2005), we consider the low chromosome numbers in these families to be a result of considerable reduction. In males of the genus *Geogarypus* (Geogarypidae), the diploid number of chromosomes ranged from 15 to 23. In the family Olpiidae, the number of chromosomes was reduced even more dramatically. Based on the study of ten species, we found the male  $2n$  to range from 7 (*Olpium pallipes*, *O. turcicum*, and *Indolpium* sp.) to 23 (*Nanolpium* sp.). This study has uncovered the lowest known number of chromosomes in pseudoscorpions, a male diploid chromosome number of seven, which is amongst the lowest ever recorded amongst arachnids. This low diploid number has only

been found in *Ariadna lateralis* (Araneae: Segestriidae), a primitive araneomorph spider exhibiting holokinetic chromosomes (Suzuki, 1954). More reduced chromosome numbers are often discovered in some mites exhibiting haplodiploidy. In mites of the suborder Mesostigmata, the diploid number can be as low as six (Oliver, 1977). This chromosome number was also discovered in one population of the scorpion *Tityus bahiensis* (Buthidae), a species noted for considerable differences of chromosome numbers among different populations studied (White, 1973). In some mites of the suborder Prostigmata, diploid numbers as low as four have been reported (Norton et al., 1993).

In spite of a considerable reduction in diploid numbers, the X0 sex chromosome system is preserved in all pseudoscorpion species examined during this study. It is the most frequently occurring mode in pseudoscorpions and has been found in various disparate families. Thus it is regarded as the ancestral system in pseudoscorpions (Troiano, 1990; Štáhlavský & Král, 2004). The extant chromosome segment on one arm of the X chromosome shows prominent positive heteropycnosis in *Geogarypus* during prophase I. We suppose that positive heteropycnosis may reflect a predominance of constitutive heterochromatin. In contrast to all other pseudoscorpions studied so far, the X chromosome of *O. pallipes* from Corfu is acrocentric. We suggest that this acrocentric sex chromosome developed by a pericentric inversion from an ancestral metacentric X chromosome. This initial condition is still preserved in the population of *O. pallipes* from Portugal (Fig. 5D). Because pericentric inversions can play an important role in reproductive isolation of putative species, especially in sex chromosomes (King, 1993), we consider that the taxonomic status of these chromosome forms of *O. pallipes* to be suspect and that more than one biological species may be present. Here, we classify them as two different chromosome races. It is therefore essential to analyse the karyotypes and morphology of other populations of *O. pallipes*, especially within the distribution range of this species between Portugal and Corfu, i.e. from Spain, South France, Italy and the Balkan Peninsula, to clarify the taxonomic status of this species.

The X chromosome exhibits heteropycnosis in some meiotic stages. In the genus *Geogarypus* we observed interspecific differences during meiotic heteropycnosis. We assume that negative heteropycnosis of the X chromosome during pachytene of *Geogarypus* sp. might reflect the metabolic activity of the sex chromosome during that stage. The relationship between the negative heteropycnosis of the sex chromosome and its metabolic activity during the early stages of spermatogenesis has been demonstrated, for example, in some grasshoppers (Church, 1979).

During the first meiotic division of males it was possible to observe a high number of chiasmata in some species; up to five per bivalent in *Indolpium* sp. Despite these findings, we did not explicitly establish a relation-

ship between the reduction of chromosome numbers and rise of chiasma frequency per bivalent (Table 3).

The transient stage between pachytene and diplotene was typically characterised by extensive decondensation of chromatin in males of geogarypids and *Calocheiridius libanoticus*. We interpret these plates as the so-called diffuse stage. The diffuse stage is often classified as a separate period of prophase during the first meiotic division (Benavente & Wettstein, 1980). Until now, it was demonstrated in various animals and plants, usually between pachytene and diplotene. Increased metabolic activity of the diffuse stage is expressed by the large size of the nucleus, lack (or imperfect morphology) of the synaptonemal complex, and considerable decondensation of chromatin. This stage apparently occurs most often in females due to the need to synthesise reserve substances in developing oocytes, as in humans; the occurrence in spermatocytes is not as common (Benavente & Wettstein, 1980). A diffuse stage is recorded in pseudoscorpions for the first time. Amongst arachnids, it has been recorded in some spiders (Suzuki, 1954; Benavente & Wettstein, 1980), ticks (Kahn, 1964), mites of the family Acaridae (Haineman & Hughes, 1970), and the harvestman *Melanopa unicolor* (Sharma & Dutta, 1959).

Previous studies on karyotype evolution within pseudoscorpions (Troiano, 1990, 1997; Štáhlavský & Král, 2004) have shown that the considerable diversity observable in their karyotypes is useful for cytotaxonomic studies. Results of the present study demonstrate that in the families Geogarypidae and Olpiidae the karyotype diversity also appears to be sufficient for taxonomic analyses. However, the data obtained are too fragmentary and thus do not allow us to reconstruct the karyotype evolution of both families. In spite of this, comparison of representatives from distant areas such as the Mediterranean, South Africa and Western Australia allows us to make some interesting conclusions. For example, to state the similarity of the karyotypes of *Horus obscurus* (South Africa) and *Calocheirus atopus* (Israel); karyotypes of these olpiids differ in the morphology of one chromosome pair only. Remarkable similarity was found in the karyotypes of *Olpium pallipes* (Mediterranean), *O. turcicus* (Israel) and *Indolpium* sp. (Western Australia). Their male karyotype consists of 7 chromosomes that exhibit similar morphology (Table 2). The chromosome complement of these species contains two large metacentric pairs and one small pair of autosomes. Apart from the fact that *O. pallipes* and *Indolpium* sp. belong to different genera, the similarity in size of both large autosome pairs appears to be significant, whereas in *O. turcicus* and *O. pallipes* it is significant for the first pair of autosomes only (Mann-Whitney U Test,  $p$ -level > 0.05) (Table 4). The small autosome pair exhibits variable morphology: it is metacentric in *O. pallipes*, subtelocentric in *Indolpium* sp. and acrocentric in *O. turcicus*. The distinctive morphology of the third autosome pair can be easily explained by pericentric inversions. Štáhlavský & Král (2004) found that pericentric inversions were frequent in the karyotype differentiation of the genus *Chthonius*. Moreover, this chro-

mosome rearrangement probably also operated in the evolution of the X chromosome in *O. pallipes*. The considerable similarities mentioned above may reflect a close relationship between these three species. To clarify the origin of the karyotypes with  $2n = 7$ , we suggest to concentrate especially on the karyotype evolution of the genera *Olpium* and *Calocheiridius*. The autosome complement of *Calocheiridius libanoticus* from Turkey is composed of three pairs of acrocentric, one pair of subtelocentric and one pair of metacentric chromosomes. The karyotype with seven chromosomes in *O. turcicus* may be derived from the karyotype of *Calocheiridius libanoticus* by two centric fusions. However, the true direction of the evolution can only be specified by further karyotype studies and/or by an analysis of molecular phylogeny.

Lastly, we emphasise the substantial differences of the karyotype of *Garypinus dimidiatus* ( $2n = 33$ , X0) from the other pseudoscorpions examined in this study. This species differs from the olpiids studied by its significantly higher chromosome number and by the unusual negative heteropycnosis of X chromosome during metaphase II. These differences tend to support the recognition of a separate family for *Garypinus* and its relatives, a status which was recently proposed by Judson (2005). However, our lack of sampling any other member of the Garypinidae mitigates against any broad, sweeping assessments of the differences observed thus far.

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