Karyotype analysis and achiasmatic meiosis in pseudoscorpions of the family Chthoniidae (Arachnida: Pseudoscorpiones)

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Karyotypes of pseudoscorpions (Arachnida, Pseudoscorpiones) are largely unknown. Here we describe for the first time karyotypes of the suborder Epiocheirata, represented by 9 European species of two genera of Chthoniidae, *Chthonius* and *Mundochthonius*. Diploid chromosome numbers of males range from 21 to 37. Karyotypes of both genera differ substantially. Acrocentric chromosomes predominate in karyotypes of the genus *Chthonius*, whereas *M. styriacus* exhibits a predominance of metacentric chromosomes. These differences suggest that the two genera belong probably to distant branches of the family Chthoniidae. It is proposed that karyotype evolution of the genus *Chthonius* was characterised by a reduction of chromosome numbers by tandem and centric fusions as well as gradual conversion of acrocentric chromosomes to biarmed ones, mostly by pericentric inversions. A tendency towards reduced chromosome numbers is evident in the subgenus *Ephippiochthonius*. All species display X0 sex chromosome system that is probably ancestral in pseudoscorpions. The X chromosome exhibits conservative morphology. It is metacentric in all species examined, and in the majority of them, a subterminal secondary constriction was found at one of its arms. In contrast to chthoniids, secondary constriction was not reported on sex chromosomes of other pseudoscorpions. Analysis of prophase I chromosomes in males revealed an achiasmatic mode of meiosis. Findings of the achiasmatic meiosis in both genera, *Chthonius* and *Mundochthonius*, indicate that this mode of meiosis might be characteristic of the family Chthoniidae. Amongst arachnids, achiasmatic meiosis has only been described in some scorpions, acariform mites, and spiders.

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Knowledge of the cytogenetics of arthropods has considerably expanded over the past few decades. Nevertheless, karyotypes of some groups are still poorly understood or are completely unavailable. This is especially true for some groups of Arachnida, one of the major classes of arthropods. While the first karyotypes of arachnids had been described by the end of 19th century (Carnoy 1885), the subsequent rate of karyological descriptions has been low, and the karyotypes of only about 1000 species of arachnids are currently known. The bulk of descriptions are confined to just a few orders, spiders (Araneae), mites (Acari), harvestmen (Opiliones), and scorpions (Scorpiones), whilst, the karyology of the other orders is largely or even completely unknown (Král 1994).

Pseudoscorpions (Pseudoscorpiones) represent the fourth largest order of arachnids. More than 3200 species are currently recognised, classified into 24 families within the suborders Epiocheirata and Iocheirata (Harvey 1992). Observations on these small predators (their body length rarely exceedes 1 cm) are relatively restricted despite their presence in a vast majority of terrestrial biotopes from all over the world where they are important predators on other small invertebrates (Weygoldt 1969).

Pseudoscorpions appear to be poorly explored karyologically, and the karyotypes of only 9 species in 3 families, Neobisiidae, Cheliferidae and Chernetidae, have been published. Basic information about pseudoscorpion chromosomes was presented by Sokolow (1926) and Boissin and Manier (1966). However, these authors focused mainly on oogenesis and spermatogenesis of certain species. Sokolow (1926) describe the karyotypes and the course of meiosis in *Neobisium carcinoides* (Neobisiidae) and *Dendrochernes cyrneus* (Chernetidae), whilst Boissin and Manier (1966) presented the karyotype of *Hysterochelifer meridianus* (Cheliferidae). Troiano (1990, 1997) presented detailed data on six Italian species of the genus *Roncus* (Neobisiidae). He also analysed the behaviour of sex chromosomes during meiosis and outlined probable modes of karyotype evolution within the genus.

The results presented by these authors suggest that the karyotypes of pseudoscorpions exhibit great diversity of diploid chromosome numbers (2n of males

range from 22 to 67), and males represent the heterogametic sex. Pseudoscorpions are further characterized by considerable interspecific variability of chromosome numbers, and occurrence of two sex chromosome systems, X0 and XY. The sex chromosomes are often distinguishable morphologically and are remarkable for their special behaviour during male meiosis. During male prophase I, they lie on the periphery of the nucleus and exhibit positive heteropycnosis.

All of the pseudoscorpion species studied to date belong to the suborder Iocheirata, whereas the karyology of pseudoscorpions of the suborder Epiocheirata has not yet been studied. In order to fill this gap, we have focused on the family Chthoniidae that is sole European group, and most diverse member, of this suborder (Harvey 1991). We here describe the karyotypes of selected species and bring proofs that the male meiosis is achiasmatic. The data obtained in this study allow us to hypothesize about possible modes of karyotype evolution within the genus *Chthonius*.

MATERIAL AND METHODS

Collection of specimens

Chthoniid pseudoscorpions were sifted from forest leaf litter using a light pad or were collected individually from the underside of stones in forested regions. The collection data for the species used in this study are presented below.

Chthonius (*Chthonius*) *diophthalmus* Daday 1888: Romania: Bihor Mts. – Cetatile Ponorului (2♂, 1♀: 17.7., 18.7.2001). *C.* (*C.*) *litoralis* Hadži 1933: Greece: Corfu-Kassiopi (6♂, 8♀: 24.4.2001, 8.5.2002). *C.* (*C.*) *orthodactylus* (Leach 1817): Czech Republic: Hněvkovice (3♂: 3.6.2001), Nuzice (3♂: 3.6.2001). *C.* (*C.*) *tenuis* L. Koch 1873: Czech Republic: Prague-Komořany (9♂, 12♀: 8.2., 8.3., 2.4.2001, 30.1., 13.10.2002).

Chthonius (*Ephippiochthonius*) *fuscimanus* Simon 1900: Czech Republic: Střemošice (11♂, 1♀: 27.5.2002). *C.* (*E.*) *tetrachelatus* (Preyssler 1790): Czech Republic: Prague-Kunratice (7♂, 2♀: 1.6.2000), Prague-Bud'ánka (5♂, 1♀: 3.5., 9.5.2001), Doubrava (3♀: 1.5.2001), Hněvkovice (2♂: 3.6.2001). *C.* (*E.*) sp. 1: Greece: Corfu – Kassiopi (10♂, 5♀: 24.4.2001, 8.5.2002). *C.* (*E.*) sp. 2: Greece: Corfu – Kassiopi (2♂$_3$ 1♀: 8.5.2002).

Mundochthonius styriacus Beier 1971: Czech Republic: Prague – Štvanice Island (17♂, 9♀: 3.6., 12.9.2001, 31.1., 24.4.2002).

Specimens were kept in glass vials with moistened cotton wool and supplied with springtails for food. We attempted to prepare chromosomes as soon as possible after collection because this was found to produce the best results. Testes of adult males collected during spring (April–May) were found to be the most suitable tissue for the karyological analysis because they contain not only spermatogonial mitoses but also various stages of meiosis. From males of *M. styriacus*, we obtained spermatogonial mitoses and stages of prophase I only. All karyotyped specimens are deposited in the collection of the first author who also determined the species.

Chromosome preparations

To prepare chromosomes from chthoniid pseudoscorpions, we modified the spreading technique described by Traut (1976). This technique is particularly convenient for the preparation of chromosomes from tiny animals. Dissection started by rupturing of the pleural wall of the opisthosoma with tweezers whilst the specimen is immersed in a hypotonic solution (0.075 M KCl) and extracting the mesenteron. The tiny gonads of chthoniids (body length 1–2 mm) were left in the body cavity to prevent their loss during the following procedure. After 10 min of hypotonic treatment, the remainder of the opisthosoma with the gonads attached was incubated in freshly prepared Carnoy fixative (ethanol: chloroform: acetic acid 6:3:1) for 20 min. During that time the fixative was changed two or three times. After fixation, the gonads were dissected and removed from the opisthosoma in a drop of 60% acetic acid on a clean microscope slide and suspended. A fine suspension was made by using a pair of sharp tungsten needles. The slide was then quickly placed on a warm histological plate (surface temperature of 40°C) and the drop of dispersed tissue was moved on the slide with the help of tungsten needle untill evaporation. Preparations were dried overnight and stained with 5% Giemsa solution in a Sörensen phosphate buffer (pH = 6.8) for 30–40 min.

For visualisation of nucleolar organizer regions (NORs), we stained preparations first by Giemsa and then by $AgNO_3$ following the 1-step method with colloidal developer (Howell and Black 1980).

Preparations were inspected in a Jenaval microscope (Carl Zeiss Jena) utilising an immersion objective and the best figures were photographed. Interestingly, mitotic chromosomes of pseudoscorpions often exhibited indistinct centromeres. Therefore, we used also metaphase II chromosomes and meiotic postpachytene bivalents to localize primary constrictions, differentiate primary and secondary constrictions, and then construct the karyotype. Except primary constrictions, sister chromatids were clearly separated during metaphase II. At postpachytene bivalents, the centromere

area was marked by a prominent knob, which enabled us to identify the centromere. Ten figures of suitable stage (mitotic metaphase, metaphase II or postpachytene) were measured and evaluated to construct the karyotype. In the majority of species, we used both metaphase II and postpachytene to compare the suitability of both variants for the construction of the karyotype. Comparison of relative chromosome lengths obtained by both methods showed that their results are comparable (Table 1). The chromosome classification system follows LEVAN et al. (1964). Chromosome lengths were calculated as a percentage of total chromosome length of the haploid set, which also includes the sex chromosome. The measured values then formed the basis for the construction of idiogrames (Fig. 5). The exact position of secondary constrictions in the karyotype could be determined only for *M. styriacus*. Other species gave only a restricted number of figures with clearly visible secondary constrictions.

RESULTS

Chthonius (Chthonius) diophthalmus Daday 1888

The male karyotype comprises 33 chromosomes, 16 autosome pairs and one X chromosome. Autosome pairs No. 1 and 9 are subtelocentric, while all others are acrocentric (Fig. 1a, 5a). Autosomes gradually decrease in size, which is similar to other species of the subgenus *Chthonius* (see below). At the metaphase II, the relative length of chromosomes range from 7.50% to 3.35% for a haploid set (Fig. 5a). The large X chromosome (25.71% of the haploid set) is metacentric (Fig. 5a). A detailed analysis of the male meiosis confirmed an X0 sex chromosome system.

Chthonius (Chthonius) litoralis Hadži 1933

All males possessed 35 chromosomes. The karyotype contains 16 pairs of acrocentric and one pair of submetacentric autosomes (No. 12) (Fig. 1b, 5b). Relative lengths of bivalents range from 6.55% to 3.26% in postpachytene. The only sex chromosome X is metacentric with one arm carrying a subterminal secondary constriction. The relative length of the X chromosome is 16.18% in postpachytene (Fig. 5b).

Chthonius (Chthonius) orthodactylus (Leach 1817)

The diploid male complement contains 33 chromosomes. At metaphase II one pair of submetacentric (No. 9) and 15 pairs of acrocentric autosomes are visible (Fig. 1c, 5c). Relative lengths of chromosomes range from 7.20% to 3.25%. The odd X chromosome is metacentric (Fig. 1c). This chromosome is the longest element of the karyotype and its one arm carries a subterminal secondary constriction. The relative length of the X chromosome is 23.21% in metaphase II.

Chthonius (Chthonius) tenuis L. Koch 1873

The diploid chromosome number of males is 35, and all autosomes are acrocentric (Fig. 1d, 1e, 5d). The relative lengths range from 6.96% to 2.75% for chromosomes in the male mitotic metaphase and from 6.70% to 2.75% for bivalents in postpachytene, respectively (Table 1, Fig. 5d). The large X chromosome is metacentric (Fig. 5d) with one arm carrying a subterminal secondary constriction. The X chromosome forms 22.07% of the haploid chromosome set at mitotic metaphase or 21.40% at metaphase II, respectively.

Chthonius (Ephippiochthonius) fuscimanus Simon 1900

The male karyotype consists of 35 chromosomes. All pairs of autosomes are acrocentric and they gradually decrease in size (Fig. 2a, 2b, 5e). The relative lengths range from 6.56% to 3.44% for bivalents in postpachytene and from 6.07% to 3.33% in metaphase II (Table 1, Fig. 5e). The X chromosome is metacentric and is the longest chromosome of the karyotype. The relative length of X chromosome is 15.76% in postpachytene and 19.35% in metaphase II (Fig. 5e).

Chthonius (Ephippiochthonius) tetrachelatus (Preyssler 1790)

A diploid male complement contains 35 chromosomes. The postpachytene karyotype contains one pair of submetacentric (No. 6) and 16 pairs of acrocentric bivalents from which one (No. 2) has the centromere in a subterminal position. This autosome is subtelocentric in metaphase II (Fig. 2c, 2d, 5f). Autosomes gradually decrease in size. Their relative lengths range from 7.07% to 2.78% for bivalents in postpachytene and from 6.13% to 2.84% for chromosomes in metaphase II, respectively (Table 1). The large X chromosome is metacentric and its one arm carries a subterminal secondary constriction. The X chromosome forms 19.72% of the total chromosome length in postpachytene and 27.80% in metaphase II (Fig. 5f).

Chthonius (Ephippiochthonius) sp. 1

The male karyotype is composed of 29 chromosomes. The karyotype consists of 11 pairs of acrocentric, two pairs of submetacentric (No. 1, 6 in postpachytene or No. 1, 5 in metaphase II, respectively) and one pair of metacentric (No. 14 in postpachytene or No. 13 in

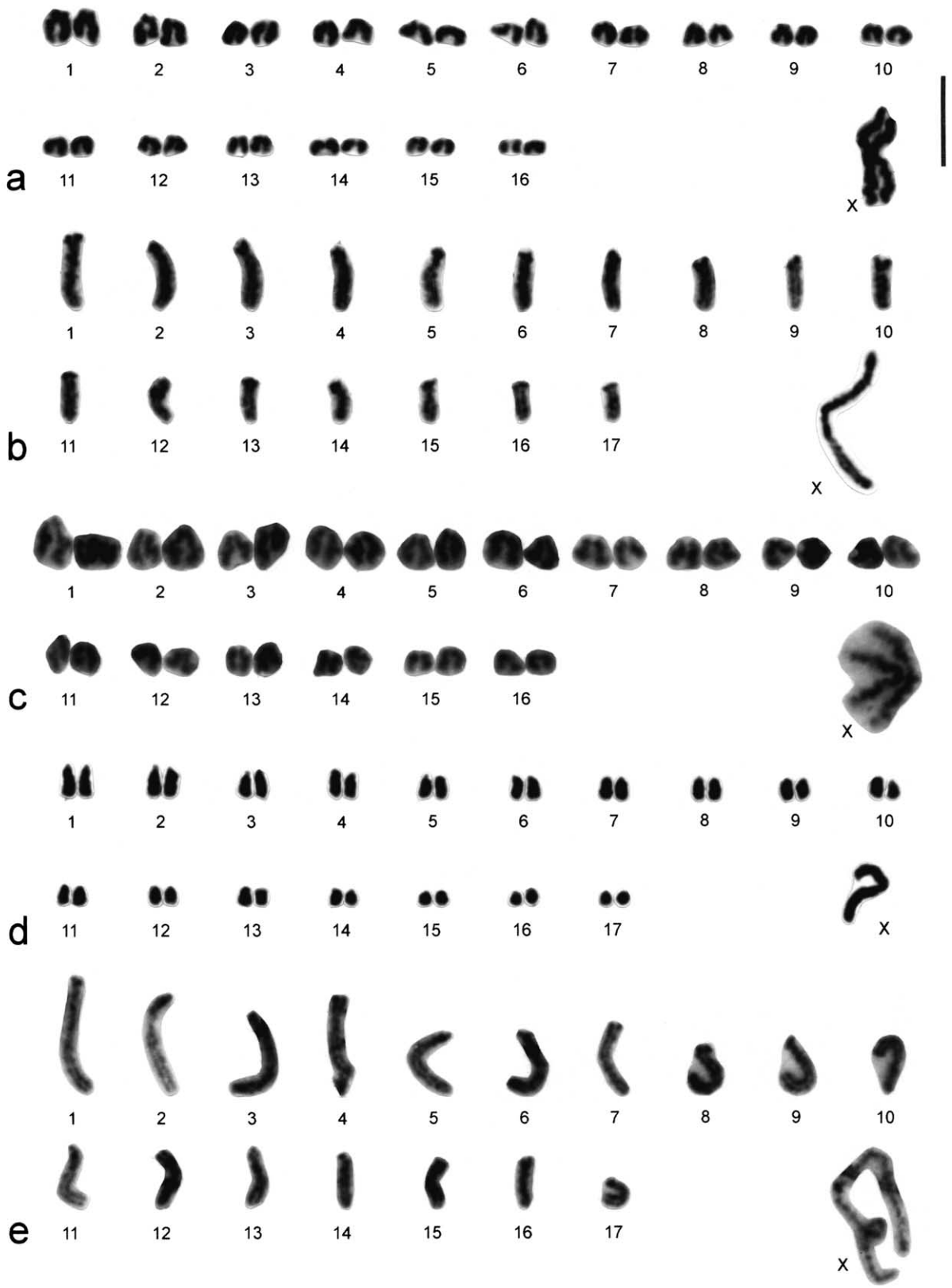

Fig. 1a–e. Karyotypes of *C.* (*Chthonius*). (**a**) *C.* (*C.*) *diophthalmus* (metaphase II), (**b**) *C.* (*C.*) *litoralis* (postpachytene), (**c**) *C.* (*C.*) *orthodactylus* (metaphase II), (**d, e**) *C.* (*C.*) *tenuis* (mitotic metaphase and postpachytene). Scale bar = 10 μm.

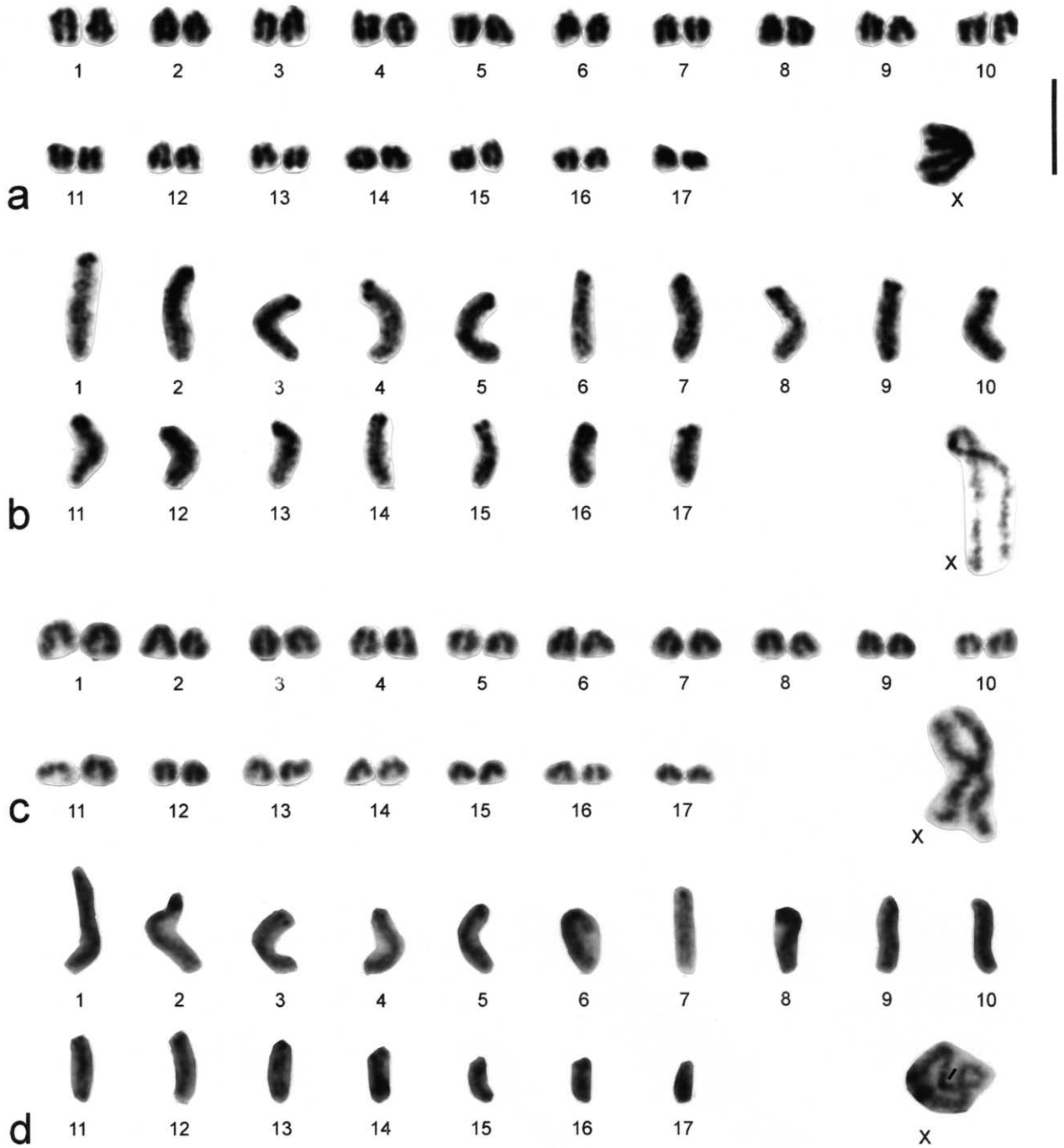

Fig. 2a–d. Karyotypes of *C.* (*Ephippiochthonius*) from central Europe. **(a, b)** *C.* (*E.*) *fuscimanus* (metaphase II and postpachytene), **(c, d)** *C.* (*E.*) *tetrachelatus* (metaphase II and postpachytene); line points knob at area of secondary constriction. Scale bar = 10 μm.

metaphase II) chromosomes (Fig. 3a, 3b, 5g). Autosomes may be divided into two size groups. The submetacentric pair No. 1 amounts nearly to the size of X chromosome (Table 1, Fig. 5g). The other autosome pairs are much shorter; their length decreases gradually from 6.96% to 2.55% in postpachytene and from 6.61% to 2.93% in metaphase II (Table 1, Fig. 5g). The large X chromosome is metacentric, and its one arm seems to carry a subterminal secondary constriction. This chromosome forms 20.92% of the haploid chromosome set in postpachytene and 22.75% in metaphase II.

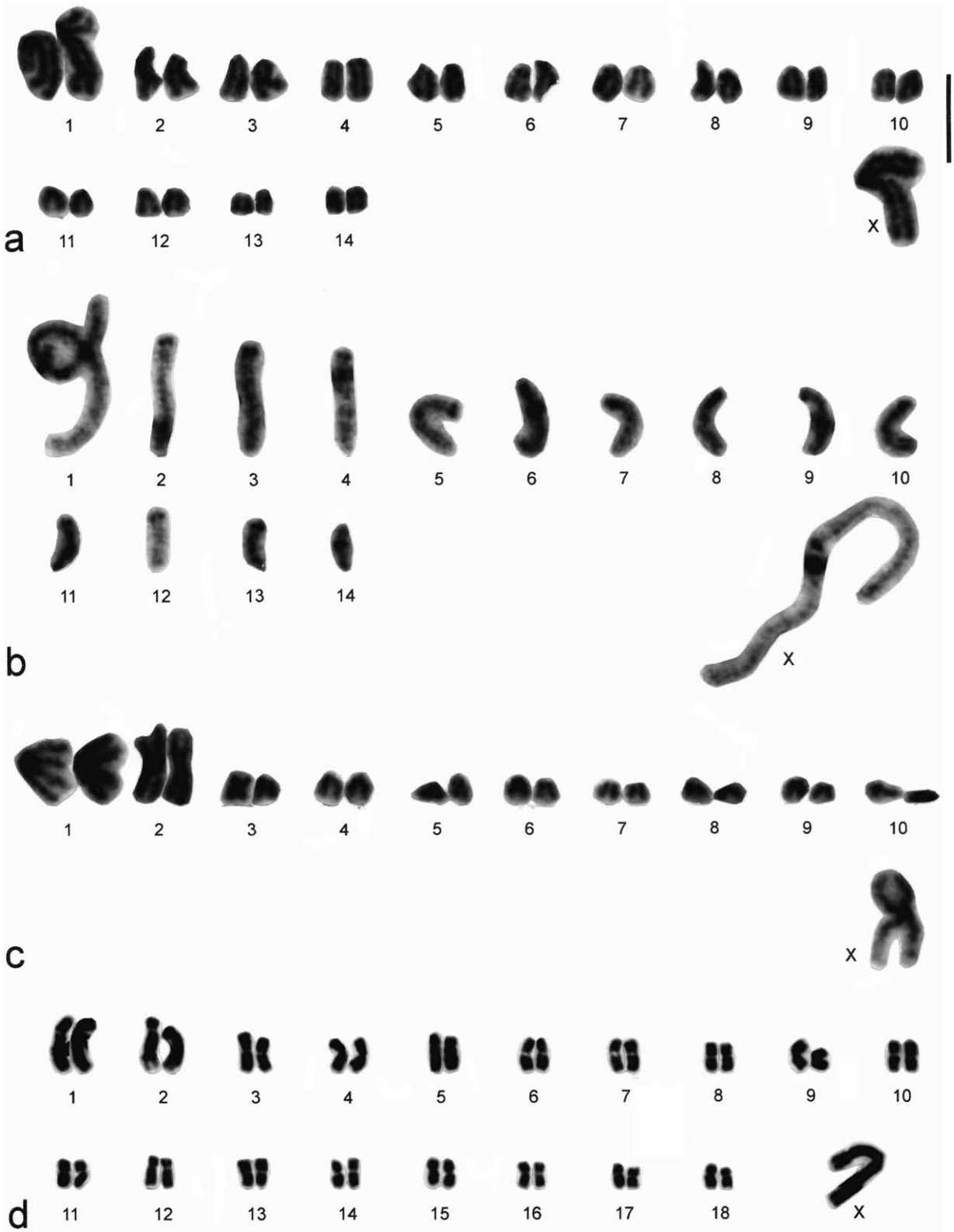

Fig. 3a–d. Karyotypes of *C.* (*Ephippiochthonius*) from Greece and *Mundochthonius.* (**a, b**) *C.* (*E.*) sp. 1 (metaphase II and postpachytene), (**c**) *C.* (*E.*) sp. 2 (metaphase II), (**d**) *M. styriacus* (mitotic metaphase). Scale bar = 10 μm.

Table 1. *C. (C.) tenuis, C. (E.) fuscimanus, C. (E.) tetrachelatus, and C. (E.) sp. 1. Comparison of chromosome relative lengths based on selected phases (mit – mitotic metaphase, ppach – postpachytene, met II – metaphase II)*

Pair No.	*C.* (*C.*) *tenuis*		*C.* (*E.*) *fuscimanus*		*C.* (*E.*) *tetrachelatus*		*C.* (*E.*) sp. 1	
	mit	ppach	met II	ppach	met II	ppach	met II	ppach
1	6,96	6,70	6,07	6,56	6,13	7,07	17,07	18,93
2	6,07	6,15	5,76	5,93	5,78	6,19	6,61	6,96
3	5,64	6,00	5,53	5,69	5,42	5,95	6,12	6,34
4	5,49	5,65	5,33	5,49	5,14	5,70	5,50	5,93
5	5,30	5,42	5,16	5,38	4,95	5,42	5,30	5,58
6	5,13	5,30	5,04	5,31	4,90	5,26	5,18	5,43
7	5,00	5,06	4,97	5,20	4,39	5,18	4,91	4,72
8	4,79	4,90	4,92	5,03	4,15	5,01	4,55	4,44
9	4,60	4,68	4,81	4,91	3,95	4,86	4,31	4,19
10	4,36	4,42	4,68	4,84	3,78	4,50	4,10	3,95
11	4,10	4,28	4,58	4,70	3,65	4,31	3,87	3,62
12	3,85	4,05	4,48	4,55	3,58	4,19	3,55	3,29
13	3,67	3,70	4,33	4,41	3,54	3,99	3,03	3,14
14	3,55	3,42	4,12	4,22	3,46	3,52	2,93	2,55
15	3,36	3,18	3,91	4,12	3,33	3,31		
16	3,18	2,94	3,61	3,75	3,20	3,06		
17	2,75	2,75	3,33	3,44	2,84	2,78		
X	22,07	21,40	19,35	15,76	27,80	19,72	22,75	20,92

Chthonius (Ephippiochthonius) sp. 2

This species has a male chromosome complement consisting of 21 chromosomes. The complement contains 2 pairs of metacentric (No. 1 and 2), 1 pair of submetacentric (No. 3), 1 pair of subtelocentric (No. 5), and 6 pairs of acrocentric autosomes (Fig. 3c, 5h). The karyotype of *Chthonius* (*E.*) sp. 2 is asymmetric, as in *Chthonius* (*E.*) sp. 1 from Corfu. Autosomes can also be divided into two size groups. The first two pairs nearly reach to the size of the X (Fig. 5h). The other autosomes are more than twice shorter than pair No. 2 and decrease gradually from 6.45% to 3.65% of the haploid set in metaphase II. The large X chromosome is metacentric, and its one arm carries a subterminal secondary constriction. The X chromosome forms 23.58% of the haploid chromosome set (Fig. 5h).

Mundochthonius styriacus Beier 1971

All males displayed a chromosome number of 37. In contrast to *Chthonius* species, metacentric chromosomes predominate in *M. styriacus.* Its karyotype consists of 14 pairs of metacentric, three pairs of submetacentric (No. 1, 5 and 12), and one pair of subtelocentric chromosomes (No. 2) (Fig. 3d, 5i). The karyotype of *M. styriacus* is slightly asymmetric. In mitotic metaphase, the length of the first and second pairs equals 9.52% and 7.52% of the total chromosome length, while the relative lengths of other pairs decrease gradually from 5.41% to 2.64% (Fig. 5i). The subtelocentric pair No. 2 bears a prominent subterminal secondary constriction on the longer arm (Fig. 3d). Comparison of mitotic metaphases of both sexes confirmed an X0 sex chromosome system (Fig. 3d, 4i), the large X chromosome being metacentric. One arm of the X chromosome carries a subterminal secondary constriction (Fig. 3d).

Analysis of meiotic division

Our study revealed an achiasmatic mode of meiosis in males of all species examined. In Fig. 4a–f, we present the sequence of achiasmatic meiotic division in chthoniids. In meiotic prophase I, diplotene and diakinesis are absent due to the lack of chiasmata. Homologous chromosomes pair perfectly during pachytene and postpachytene (Fig. 4a–c). Postpachytene bivalents bear no chromomeres or knobs with the exception of a prominent centromeric knob. In some species (e.g. *C.* (*E.*) *tetrachelatus*), there is also a second, smaller knob associated apparently with a secondary constriction (Fig. 2d). Staining of meiotic phases by silver nitrate revealed that the nucleolus only disappears at the end of postpachytene (not shown). During late postpachytene and metaphase I, centromeric areas of homologous chromosomes start to separate (Fig. 4d). During that time, sister chromatids of chromosomes are starting to be visible. Apparently, centromeric areas separate more notably in *Mundochthonius* than in *Chthonius* (Fig. 4h).

In males of all species, the univalent X chromosome exhibits positive heteropycnosis during early prophase I (leptotene-pachytene) forming a prominent spherical body on the periphery of the nucleus (Fig. 4a). The

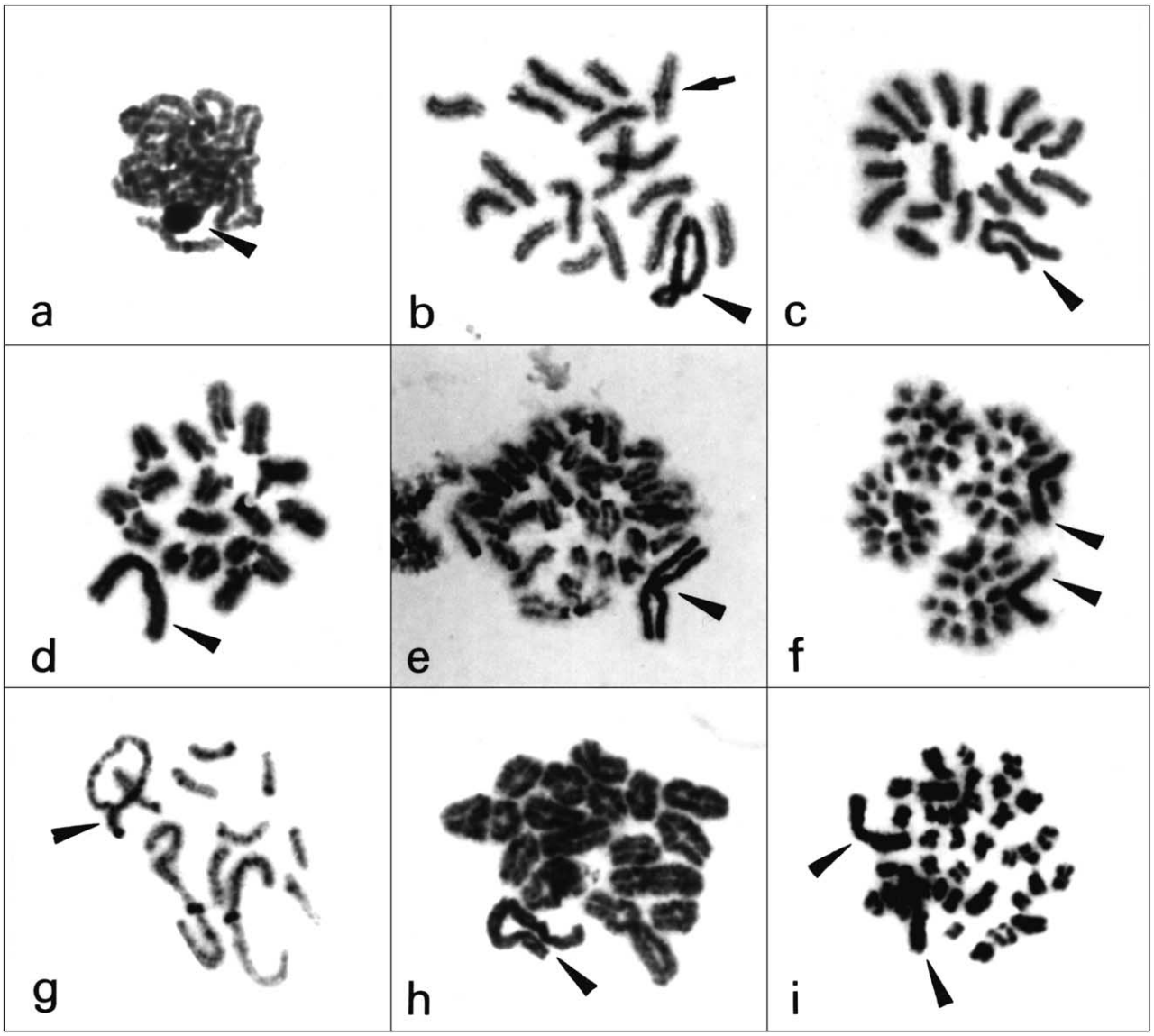

Fig. 4a–i. (**a–f**) Sequence of achiasmatic meiosis in chthoniid *C.* (*C.*) *litoralis*; arrowhead points to X chromosome. (**a**) pachytene; X chromosome forms heteropycnotic sex vesicul, (**b**) early postpachytene; heteropycnotic X chromosome is characterized by pairing of the distal regions of arms. Arrow indicates centromeric knob of submetacentric chromosome pair, (**c**) late postpachytene; arms of the X chromosome are perfectly separated. Centromere regions of homologous chromosomes start to separate, (**d**) metaphase I; note continued separation of centromeric regions as well as individualization of sister chromatids, (**e**) metaphase II, (**f**) anaphase II; X chromosome exhibits positive heteropycnosis during metaphase and anaphase II, (**g**) *C.* (*E.*) sp. 2, early postpachytene; arms of X chromosome pair each other by proximal as well as distal parts, (**h**) male of *M. styriacus*, postpachytene, (**i**) female of *M. styriacus*, oogonial metaphase; arrows indicate two X chromosomes. Scale bar = 10 μm.

body disappears at the end of pachytene. During early postpachytene, the X chromosome unrolls gradually, while the distal ends of both arms remain associated (Fig. 4b). In *C.* (*E.*) sp. 2 from Corfu, the proximal regions of the arms close to the centromere are also associated (Fig. 4g). Heteropycnosis of the X chromosome disappears usually during the transition from pachytene to postpachytene. In *C.* (*E.*) sp. 1 only, the sex chromosome exhibits positive heteropycnosis from leptotene to pachytene but negative heteropycnosis during postpachytene. In *C.* (*C.*) *litoralis*, positive heteropycnosis of the X chromosome vanishes during postpachytene (Fig. 4b–c) but it emerges again during metaphase and anaphase of the second meiotic divi-

sion (Fig. 4e–f). In males of all species, sister chromatids of the X chromosome separate slightly earlier than those of autosomes during metaphase II.

DISCUSSION

This study presented, for the first time, karyotypes of pseudoscorpions of the family Chthoniidae. In males, the diploid number of chromosomes ranged from 21 to 37. All species display an X0 sex chromosome system. Interestingly, the genera *Chthonius* and *Mundochthonius* differ substantially in their chromosome morphology. Acrocentric chromosomes predominate in karyotypes of the genus *Chthonius*. On the contrary, the karyotype of *M. styriacus* is distinguished by a predominance of metacentric chromosomes. We suggest that large karyotype differences between the genera *Chthonius* and *Mundochthonius* may reflect that they belong to distant evolutionary branches of the family Chthoniidae.

Representatives of the family Chthoniidae apparently do not differ in chromosome numbers and morphology as much as the previously studied species of the family Neobisiidae (Troiano 1990, 1997). Nevertheless, results of our study demonstrate that also in chthoniid pseudoscorpions diversity in karyotypes appears to be sufficient to be useful for cytotaxonomic studies. The karyological data obtained allow us to propose a scheme of karyotype evolution within the genus *Chthonius* (Fig. 5j). Our ongoing study indicates that the postulated scheme could be used for reconstruction of morphological evolution in chthoniids.

We assume that the ancestral male karyotype of the genus *Chthonius* consisted of 17 acrocentric pairs of autosomes that gradually decreased in size and metacentric X chromosome. The proposed original condition is still retained in some species of the subgenera *Chthonius* and *Ephippiochthonius*, namely *C.* (*C.*) *tenuis* and *C.* (*E.*) *fuscimanus* (Fig. 5d, 5e). We suggest that the subsequent karyotype evolution in the genus *Chthonius* was characterised by a reduction of chromosome numbers by tandem and centric fusions as well as gradual conversion of acrocentric chromosomes to biarmed ones. We suppose that biarmed chromosomes originated mostly by pericentric inversions and/or by accumulation of constitutive heterochromatin into short arms of chromosomes. Karyotypes of *C.* (*C.*) *litoralis* and *C.* (*E.*) *tetrachelatus* were derived from the ancestral condition by one or two pericentric inversions, respectively (Fig. 5b, 5f). The karyotypes of *C.* (*C.*) *orthodactylus* and *C.* (*C.*) *diophthalmus* represent the first step of reduction of chromosome number. In this case, the diploid number was decreased to 33 chromosomes probably by one tandem fusion (Fig. 5a, 5c). This change was accompanied by the conversion of one (*C.* (*C.*) *orthodactylus*) or two (*C.* (*C.*) *diophthalmus*) acrocentric pairs to biarmed ones. Interestingly, the trend to a reduction of chromosome numbers is expressed also in the karyotype evolution of neobisiid pseudoscorpions of the genus *Roncus*. In this case, the reduction of chromosome numbers was realised predominantly by centric fusions (Troiano 1990).

The tendency towards reduced chromosome numbers is more pronounced in the subgenus *Ephippiochthonius*. The karyotype of *C.* (*E.*) sp. 1 from Corfu is possible to derive from the karyotype of *C.* (*E.*) *fuscimanus* as follows. The formation of the largest, submetacentric chromosome pair in the karyotype of *C.* (*E.*) sp. 1 included centric fusion between two acrocentric chromosome pairs and series of two tandem fusions that were concerned in the formation of long arm of submetacentric chromosome. These changes were accompanied by a pericentric inversion of two acrocentric chromosome pairs (Fig. 5g). A reduction of diploid chromosome numbers culminated in *C.* (*E.*) sp. 2 from Corfu. In this species, the diploid number of males was reduced to 21 by the formation of a further large biarmed chromosome that originated by a similar mode as the large submetacentric chromosome in *C.* (*E.*) sp. 1 (Fig. 5h). At present, *C.* (*E.*) sp. 2 is the pseudoscorpion with the lowest known 2n.

All karyotyped chthoniids possess a X0 sex chromosome system that is more frequent and probably more primitive than the XY system in pseudoscorpions (Troiano 1990). The X chromosome exhibits conservative morphology in all species examined in this study. This metacentric chromosome is always the longest chromosome in the karyotype. Progressive decreasing of chromosome number in the genus *Chthonius* apparently did not substantially influence the morphology and behaviour of the X chromosome. We suggest that the partial autosynapsis of the X chromosome in *C.* (*E.*) sp. 2 during postpachytene forms an interesting exception. In this species, both arms of the X chromosome pair each other in the proximal regions during postpachytene. We believe that such unusual pairing may reflect homology of involved parts of the arms as a consequence of a rearrangement.

In the majority of species examined, we observed subterminal secondary constriction not stained with Giemsa at one arm of X chromosome. We suppose that this constriction is most probably related to the nucleolar organiser region (NOR). Besides the X chromosome, we have often observed secondary con-

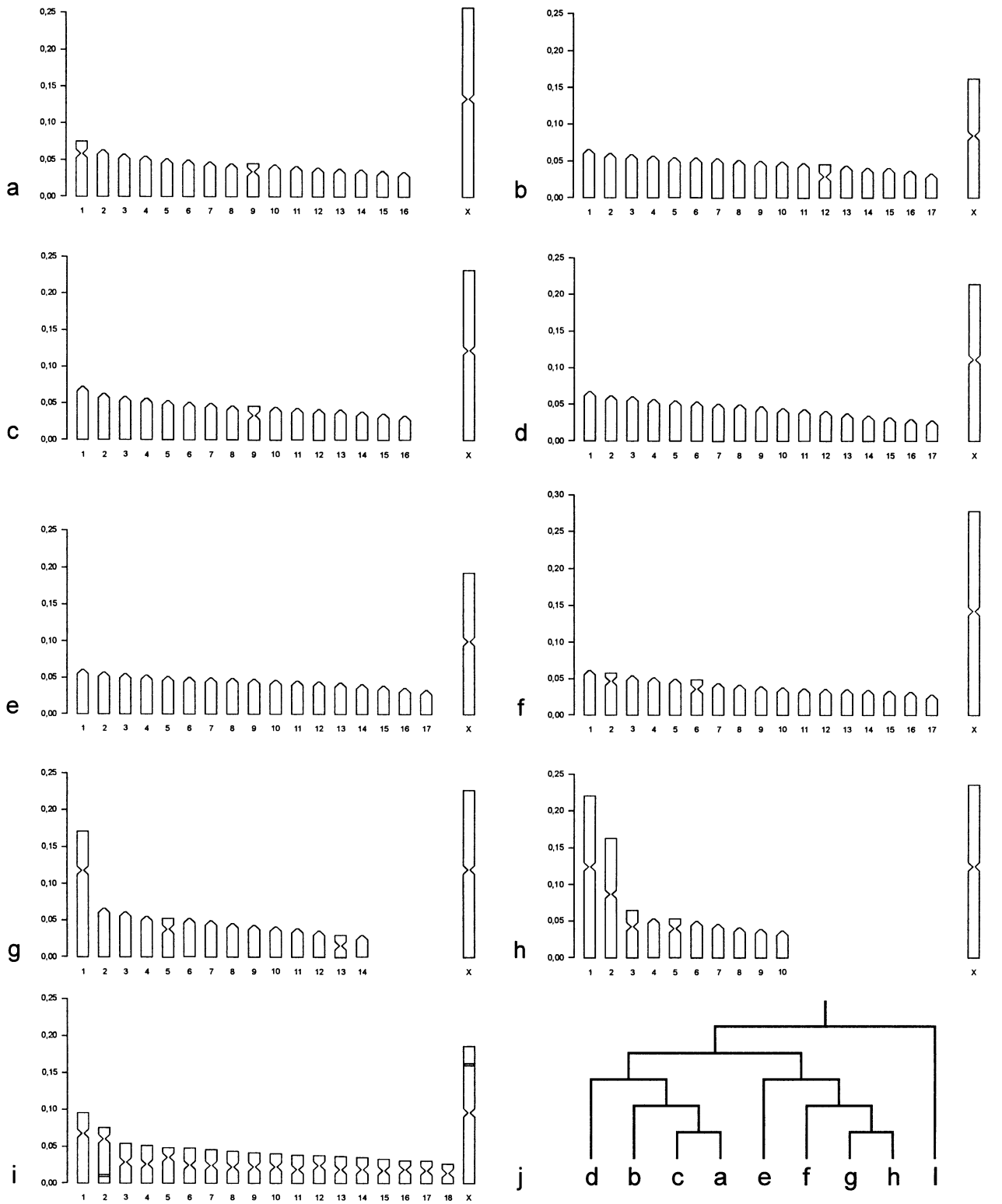

Fig. 5a–j. (**a–i**) Idiograms of studied chthoniids (y axis – % of the total chromosome length). (**a**) *C.* (*C.*) *diophthalmus* (metaphase II), (**b**) *C.* (*C.*) *litoralis* (postpachytene), (**c**) *C.* (*C.*) *orthodactylus* (metaphase II), (**d**) *C.* (*C.*) *tenuis* (postpachytene), (**e**) *C.* (*E.*) *fuscimanus* (metaphase II), (**f**) *C.* (*E.*) *tetrachelatus* (metaphase II), (**g**) *C.* (*E.*) sp. 1 (metaphase II), (**h**) *C.* (*E.*) sp. 2 (metaphase II), (**i**) *M. styriacus* (mitotic metaphase); note subterminal secondary constriction on autosome pair No. 2 and sex chromosome, (**j**) cladogram based on proposed scheme of karyotype evolution.

strictions on several pairs of autosomes. These constrictions also had a subterminal location. Unfortunately, our attempts to visualise NORs in the karyotypes using silver nitrate failed. In contrast to chthoniids, secondary constrictions were not reported on the sex chromosomes of other pseudoscorpions (Sokolow 1926, Boissin and Manier 1966, Troiano 1990, 1997, Štáhlavský 2000).

From an evolutionary point of view, chthoniids are considered to be within the most plesiomorphic branch of the order Pseudoscorpiones. This placement (Harvey 1992) seems to be well supported by their morphology and copulatory behaviour (Weygoldt 1969). The high antiquity of chthoniid pseudoscorpions is documented also by the fact that oldest known pseudoscorpion *Dracochela deprehendor* (Dracochelidae) from the Middle Devonian of Gilboa (USA) (Schawaller et al. 1991) is placed into the superfamily Chthonioidea (Harvey 1992). We propose that the ancestral pseudoscorpion karyotype was possibly composed of a high number of chromosomes and included an X0 sex chromosome system. In contrast to that, the karyotypes of examined chthoniids seem to be rather derived, exhibiting a quite low number of chromosomes and achiasmatic meiosis. To explain this observed discrepancy between morphological and karyological data, it will be necessary to study the karyotypes of other chthoniid groups to establish fundamental traits of karyotype evolution in the superfamily Chthonioidea.

Analysis of male meiosis revealed absence of diplotene and diakinesis due to the lack of chiasmata, i.e. achiasmatic meiosis. This form of meiosis is notable for an absence of chiasmata and crossing-over. In the majority of cases, the abolition of chiasmata is confined to one sex, usually heterogametic (White 1973). Achiasmatic meiosis of chthoniid pseudoscorpions adopts a classical course. Interestingly, postpachytene bivalents bear no knobs or chromomeres except the prominent knob at the centromere area. In some species, the region of secondary constriction is also marked by a knob (for example *C.* (*E.*) *tetrachelatus*, Fig. 2d). Although we were not able to obtain meiotic stages subsequent to the pachytene from females, we suppose that achiasmatic meiosis is restricted only to males in the studied species. Our findings of the achiasmatic meiosis in both the genus *Chthonius* and *Mundochthonius* which belong probably to different evolutionary lineages of the family Chthoniidae indicate that this form of meiosis might be characteristic of all representatives of the family Chthoniidae. Achiasmatic meiosis has originated independently in various groups all over the animal kingdom (except for mammals) and in some plants (White 1973). Among arachnids, this mode of meiosis has been revealed in the majority of scorpions studied to date (de Toledo 1941, Venkatanarasimhaiah 1965, Shanahan and Hayman 1990), some acariform mites (Keyl 1957), and spiders of the families Dysderidae and Segestriidae (Benavente and Wettstein 1980, Rodríguez Gil et al. 2002). Except for some scorpions, all of these groups exhibit holokinetic (holocentric) chromosomes (de Toledo 1941, Oliver 1977, Benavente and Wettstein 1980), while chthoniid pseudoscorpions possess normal (i.e. monocentric) chromosomes. We suggest that the origin of achiasmatic meiosis in chthoniids could be facilitated by a low number of chiasmata per bivalent in their chiasmatic ancestor. Low numbers of chiasmata have been found in the majority of karyotyped pseudoscorpions (Sokolow 1926, Boissin and Manier 1966, Troiano 1990, 1997, Štáhlavský 2000).

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