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Karyotype Study on Pseudoscorpions of the Genus *Lasiochernes* Beier (Pseudoscorpiones, Chernetidae)

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Karyotypes of the genus *Lasiochernes* (Pseudoscorpiones, Chernetidae) are studied for the first time. The diploid chromosome numbers of males were found to be 2n=61 in *L. pilosus*, 2n=69 in *L. siculus* and 2n=73 in *L. cretonatus*. Karyotypes of all species mainly consist of biarmed chromosomes; the sex chromosome system is X0. Remarkably, the X chromosome displays partial (*L. cretonatus*), or even total (*L. pilosus*), negative heteropycnosis during the spermatogonial metaphase.

Key words: Pseudoscorpiones, Chernetidae, *Lasiochernes*, karyotype.

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Karyotypes of some animal groups, such as vertebrates and insects, are relatively well known. In contrast, there are still many groups for which the chromosomes are largely or even completely unknown. This is true also for certain arachnid orders, including pseudoscorpions.

More than 3200 species of pseudoscorpions, placed into 24 families, have been described (HARVEY 1992). Despite this diversity, karyotypes have only been presented for 20 species of pseudoscorpions in four families. Basic information about the chromosomes of *Neobisium carcinoides* (Hermann, 1804) (Neobisiidae), *Dendrochernes cyrneus* (L. Koch, 1873) (Chernetidae) and *Hysterochelifer meridianus* (L. Koch, 1873) (Cheliferidae) were obtained during studies of spermatogenesis by SOKOLOW (1926) and BOISSIN and MANIER (1966). Further karyotypic studies of pseudoscorpions have only appeared during the past fifteen years. TROIANO (1990, 1997) described karyotypes of six *Roncus* species (Neobisiidae) from northern Italy (2n ranging from 22 to 52). The chromosomes of *Chernes hahnii* (C.L. Koch, 1839) and *Ch. similis* Beier, 1929 (Chernetidae) were described by ŠŤÁHLAVSKÝ (2000). Karyotypes of nine European chthoniids were analysed by ŠŤÁHLAVSKÝ and KRÁL (2004). From this limited data it may be concluded that pseudoscorpions exhibit great diversity in both diploid chromosome numbers (2n♂ ranges from 22 to 67) and in the morphology of chromosomes. TROIANO (1990, 1997) and ŠŤÁHLAVSKÝ and KRÁL (2004) emphasized the importance of large karyotype diversity for the solution of frequent taxonomic problems in pseudoscorpions, which arise because of their relatively uniform external morphology.

The present study concerns the karyology of three species of *Lasiochernes*, a genus of the family Chernetidae. Karyotypes and the course of meiosis are described, including the behaviour of sex chromosomes. The family Chernetidae is the largest family of pseudoscorpions, with more than 600 described species in 110 genera (HARVEY 1991). In spite of this, karyotypes of only three species have previously been studied (*Dendrochernes cyrneus*, *Chernes hahnii* and *C. similis*) (SOKOLOW 1926; ŠŤÁHLAVSKÝ 2000). Up till now, ten species of the genus *Lasiochernes* have been described. Two of these are from Central Africa, while *L. pilosus* is distributed in western Europe to Yugoslavia and the other species all occur in the Mediterranean region. They are rarely collected and are usually associated with the nests of small mammals or are found in caves (Henderickx 1998).

The data presented here can be used not only for the cytotaxonomy of chernetids but also contribute to a better understanding of the karyotype diversity of pseudoscorpions.

Material and Methods

Only a few individuals were examined because living *Lasiochernes* specimens are hard to obtain. Collection and species determination was performed by the second author. Specimens are deposited in the collections of the first two authors. Data on karyotyped specimens, namely localities, number and sex of analysed specimens as well as date of collection, are as follows: *Lasiochernes pi-*

Table 1

Relative length (% TCL) and centromeric index (AR) of particular chromosome pairs (mit – mitotic metaphase, met II – metaphase II)

Pair No.	*L. pilosus*				*L. siculus*		*L. cretonatus*	
	mit		met II		mit		mit	
	% TCL	AR	% TCL	AR	% TCL	AR	% TCL	AR
1	5.03	1.18	4.82	1.15	4.10	1.24	3.73	1.83
2	4.50	1.17	4.55	1.68	3.91	2.90	3.56	3.49
3	4.46	1.38	4.21	1.24	3.72	3.99	3.38	2.72
4	3.84	3.87	4.15	1.22	3.49	1.65	3.21	3.45
5	3.70	1.37	3.89	1.56	3.41	2.61	3.13	3.33
6	3.70	2.14	3.90	2.10	3.32	2.07	3.08	2.17
7	3.56	1.10	3.65	3.34	3.24	3.04	2.98	2.73
8	3.34	2.71	3.58	1.95	3.15	2.63	2.95	3.03
9	3.33	5.07	3.50	3.98	3.07	3.46	2.94	3.30
10	3.24	2.47	3.39	2.01	2.97	2.37	2.92	1.45
11	3.17	1.52	3.28	1.90	2.92	2.98	2.83	2.99
12	3.12	3.15	3.25	4.15	3,00	1.47	2.81	2.05
13	3.11	3.58	3.20	1.11	2.84	1.94	2.77	3.33
14	3.08	3.75	3.19	3.61	2.76	2.33	2.74	2.78
15	2.93	3.33	3.14	1.65	2.75	3.96	2.73	3.15
16	2.91	1.34	3.06	2.21	2.69	2.21	2.69	1.75
17	2.82	3.53	2.87	5.18	2.68	2.10	2.65	2.54
18	2.79	1.70	2.87	2.49	2.58	1.56	2.60	3.77
19	2.71	3.35	2.86	4.78	2.58	2.51	2.59	2.27
20	2.68	1.22	2.77	1.46	2.61	2.22	2.49	3.26
21	2.67	1.90	2.67	1.45	2.54	-	2.48	2.94
22	2.56	2.74	2.70	2.85	2.50	2.30	2.41	2.15
23	2.53	2.20	2.61	2.34	2.49	2.73	2.40	1.51
24	2.51	1.29	2.60	1.32	2.43	2.02	2.34	2.39
25	2.48	3.29	2.45	2.35	2.37	2.29	2.27	1.94
26	2.37	1.21	2.27	1.40	2.36	3.16	2.23	1.96
27	2.20	1.60	2.25	3.17	2.28	2.28	2.22	3.26
28	2.13	1.26	2.23	1.47	2.21	2.37	2.14	2.21
29	2.09	2.27	1.96	1.32	2.13	2.44	2.06	1.59
30	1.73	1.40	1.89	1.09	2.10	1.85	1.97	2.00
31					1.98	2.78	1.96	1.80
32					1.92	2.33	1.95	2.75
33					1.87	–	1.80	1.58
34					1.84	1.81	1.71	1.33
35							1.64	–
36							1.58	1.60
X	8.71	1.11	5.86	1.14	7.23	1.08	8.05	1.15

losus (Ellingsen, 1910): Belgium, Brussels, Zoniënwoud, 24.4.2001, (1♂); *L. siculus* Beier, 1961: Italy, Sicily, Pantalia, Grotta dei Pipistrelli, 13.5.2003 (2 ♂, 1♀); *L. cretonatus* Henderickx, 1998: Greece, Crete, Azogires, 13.4.2002 (1♀, 1 male tritonymph).

The chromosome preparations were made from gonads using the modified spreading technique described by TRAUT (1976), which gives good results for small invertebrates. Briefly, gonads were hypotonized for 10 min (0.075 M KCl) and then fixed in fresh Carnoy fixative (ethanol: chloroform: acetic acid 6:3:1) for 20 min. After fixation, tissue was suspended in a drop of 60% acetic acid on a clean slide using tungsten needles. The drop of dispersed tissue was placed on a histological plate (surface temperature 40°C) and moved around the slide using a needle. Chromosome preparations were dried and stained by a 5% Giemsa solution in Sörensen phosphate buffer (pH=6.8) for 40 min.

The chromosome classification system follows LEVAN *et al.* (1964). Evaluation of chromosome morphology was based on ten mitotic metaphases and, in *L. pilosus*, an additional ten meiotic metaphases II (Table 1). Metaphase II permits the recognition of the position of the centromere and distinguishes primary and secondary constrictions of chromosomes much more precisely. Chromosome lengths were calculated as a percentage of total chromosome length of the haploid set (% TCL), which also includes the sex chromosome. In *L. pilosus* and *L. siculus* the course of meiosis was also studied.

Results

Lasiochernes pilosus (Ellingsen, 1910)

The diploid chromosome number of the male is 61. Male metaphase II shows 14 metacentric, 9 submetacentric and 7 subtelocentric pairs of autosomes (Table 1, Fig. 1). Only small differences of chromosome morphology were found in the male mitotic metaphase, which consists of 14 metacentric, 7 submetacentric and 9 subtelocentric pairs of autosomes (Table 1, Fig. 2). In both cases, autosomes gradually decrease in size. The relative lengths of autosomes range from 4.82% to 1.88% in metaphase II and from 5.03% to 1.73% in male mitotic metaphase (Table 1).

Analysis of male meiosis indicates an X0 sex chromosome system. The large X chromosome is metacentric and forms 5.86% of the haploid set in metaphase II or 8.71% of this set in mitotic metaphase. One arm of the X chromosome shows a subterminal secondary constriction. The X chromosome of *L. pilosus* usually exhibits the same intensity of pycnosis as the autosomes in prometaphase and early metaphase of mitosis. In the transition to late mitotic metaphase, the arm of the sex chromosome without the secondary constriction displays partial negative heteropycnosis (Fig. 2). Finally, the whole X chromosome exhibits negative heteropycnosis (Fig. 5) in late metaphase when sister chromatids are well separated. During diplotene, all chromosomes seem to be isopycnotic (Fig. 6). Heteropycnosis of the X chromosome reappears during the second meiotic division. The X chromosome exhibits a distinct positive heteropycnosis at metaphase II (Fig. 7). During diplotene, the majority of the bivalents are unichiasmatic. Only two (67%) or three bivalents (17%) usually exhibit two chiasmata (number of observed figures = 20).

Lasiochernes siculus Beier, 1961

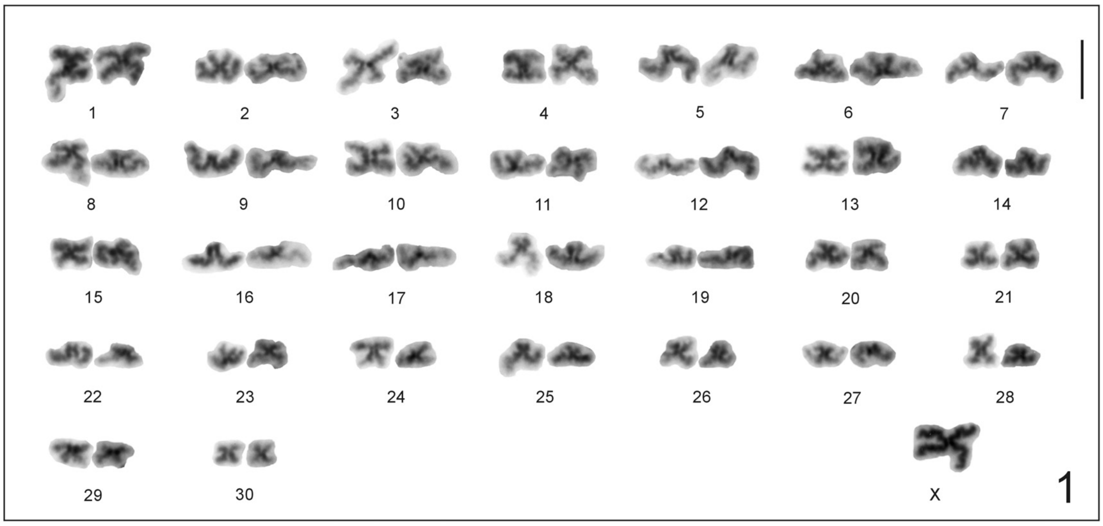
The diploid chromosome number of the male is 69. Mitotic metaphase consists of 4 metacentric, 23 submetacentric, 5 subtelocentric and 2 acrocentric pairs of autosomes (Table 1, Fig. 3). The autosomes decrease gradually in size. The relative lengths of autosomes range from 4.10% to 1.84% of the haploid set in mitotic metaphase (Table 1).

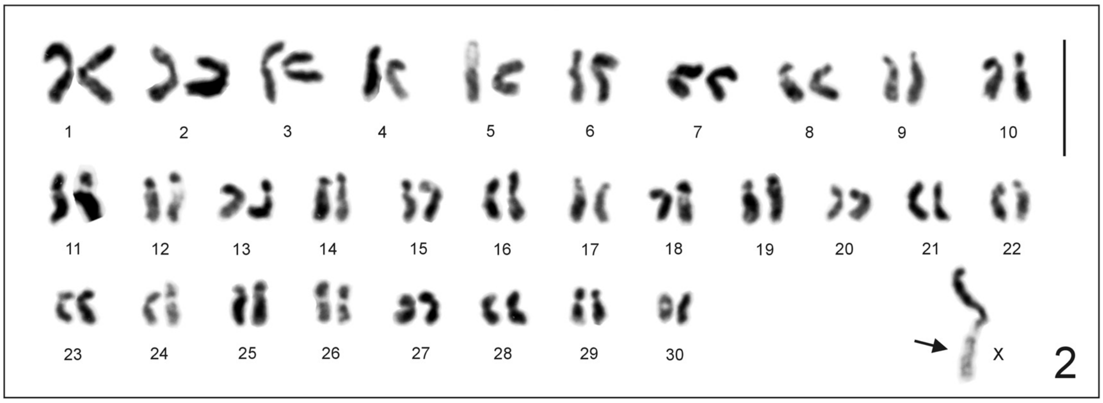
The X chromosome is metacentric and is the longest chromosome in the karyotype, its relative length being 7.23% of the haploid set. All chromosomes are isopycnotic in the observed figures of mitotic division. During diplotene, the sex chromosome sometimes displays isopycnosis in some cases, otherwise it is almost negatively heteropycnotic. During this stage, all bivalents are unichiasmatic.

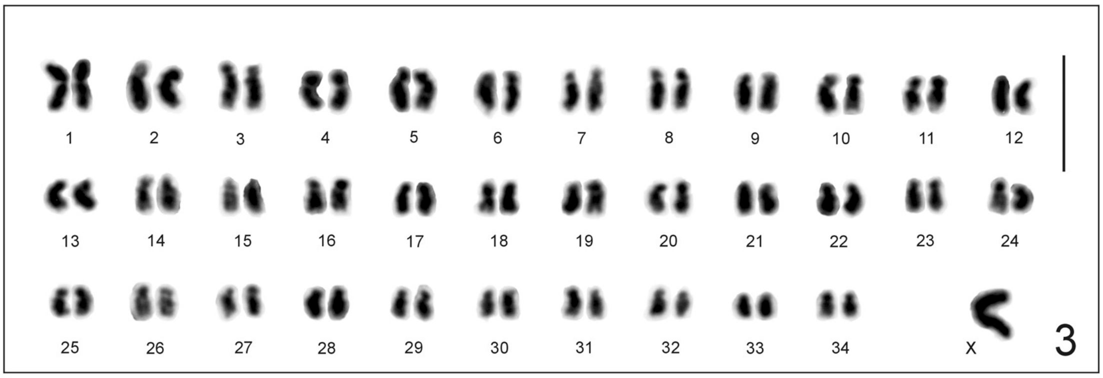
Lasiochernes cretonatus Henderickx, 1998

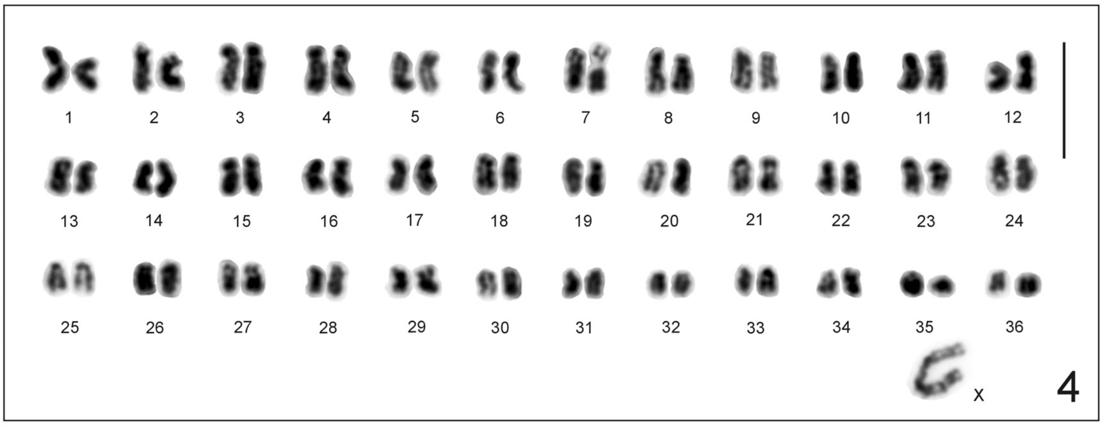
The diploid complement comprises 73 chromosomes in the male and 74 chromosomes in the female. The male complement contains 5 metacentric, 20 submetacentric, 10 subtelocentric and 1 acrocentric pairs of autosomes (Table 1, Fig. 4). The relative lengths of the autosomes decrease gradually from 3.73% to 1.58% of the haploid set in mitotic metaphase.

L. cretonatus possesses the X0 type of sex chromosome system. The metacentric X chromosome is the longest element of the karyotype (more than twice as long as the longest autosome pair) and forms 8.05% of the haploid set. One arm of the X chromosome contains a subterminal secondary constriction whereas a distal part of the second arm is often negatively heteropycnotic during mitotic metaphase.

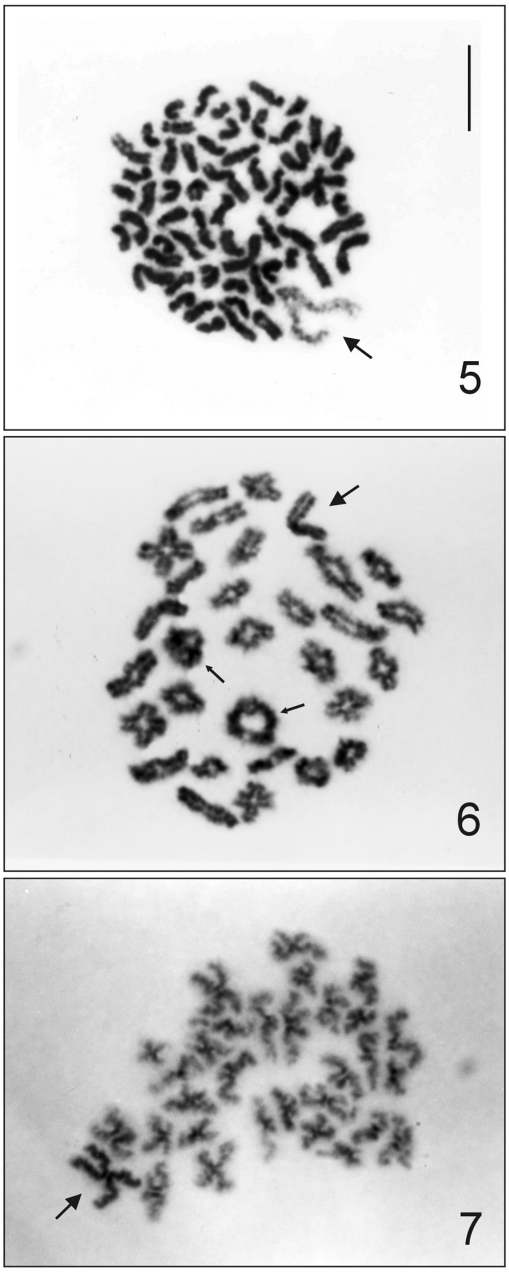








Figs 1-4. Male karyotypes of *Lasiochernes*. Fig. 1. *L. pilosus* (metaphase II). Fig. 2. *L. pilosus* (metaphase of spermatogonial mitosis). Note negative heteropycnosis of one arm of the X chromosome (arrow). Fig. 3. *L. siculus* (metaphase of spermatogonial mitosis). Fig. 4. *L. cretonatus* (metaphase of spermatogonial mitosis). Bars = 10 μm.



Figs 5-7. *Lasiochernes pilosus*. Fig. 5. Mitotic metaphase; X chromosome exhibits negative heteropycnosis (arrow). Fig. 6. Diplotene; note X chromosome (big arrow) and bivalents with two chiasmata (small arrows). Fig. 7. Metaphase II; X chromosome exhibits positive heteropycnosis (arrow). Bar = 10 μm.

Discussion

Despite the small number of karyotyped species, the data presented here demonstrate considerable diversity of chromosome numbers and the predominance of biarmed chromosomes in the karyotypes of chernetid pseudoscorpions. The latter character is typical for all studied species of pseudoscorpions except the genus *Chthonius*, in which karyotypes mainly consist of acrocentric chromosomes (ŠŤÁHLAVSKÝ & KRÁL 2004). Male diploid numbers of chernetids range from 49 in *Chernes hahnii* (ŠŤÁHLAVSKÝ 2000), to 73 in *Lasiochernes cretonatus*. The average male chromosome number in karyotypes of chernetid pseudoscorpions is higher (2n♂=62) than those of the families Neobisiidae (2n♂=39; range 22-67) (SOKOLOW 1926; TROIANO 1990, 1997) and Chthoniidae (2n♂=33; range 21-37) (ŠŤÁHLAVSKÝ & KRÁL 2004). At present, *L. cretonatus* is the pseudoscorpion with the highest known diploid number of chromosomes.

The chernetid species studied to date are characterized by an X0 sex chromosome system, which is more frequent and probably more primitive than the XY system in pseudoscorpions (TROIANO 1990). Besides the *Lasiochernes* species studied here, the X0 type of sex chromosome system is known also in *Chernes* species (ŠŤÁHLAVSKÝ 2000). The record of an $X_1X_2 0$ sex chromosome system in *Dendrochernes cyrneus* (SOKOLOW 1926) was probably a misinterpretation of the X0 system (TROIANO 1990); our own unpublished observations confirm an X0 system in this species. The X chromosome of studied species exhibits a conservative morphology. Like in the majority of pseudoscorpions analysed, this chromosome is metacentric and the longest in the karyotype. One arm of the X chromosome bears a subterminal secondary constriction. This constriction is probably related to the nucleolar organizer region (NOR). A similar secondary constriction has been reported on the X chromosome of chthoniid pseudoscorpions (ŠŤÁHLAVSKÝ & KRÁL 2004), but not in other pseudoscorpions (SOKOLOW 1926, BOISSIN & MANIER 1966, TROIANO 1990, 1997).

The negative heteropycnosis of the X chromosome at spermatogonial metaphases of *L. pilosus* and *L. cretonatus* is also of special interest. Among arachnids, heteropycnosis of sex chromosome(s) during spermatogonial mitosis is relatively common in spiders, but it has not been found in any other order (KRÁL 1994a, b).

The diversity in karyotypes of *Lasiochernes* appears to be sufficient for their use in cytotaxonomic studies. The number of species studied is not yet sufficient for the construction of detailed hypothesis about phylogeny of the genus *Lasiochernes*, as has been done in the better known genera *Roncus* (TROIANO 1997) and *Chthonius* (ŠŤÁHLAVSKÝ & KRÁL 2004). However, this study indicates that *L. siculus* and *L. cretonatus* are more closely related to each other than to *L. pilosus*. The karyotypes of these two species are similar and contain a large number of submetacentric chromosomes, while that of *L. pilosus* differs in having a higher number of metacentric chromosomes (Table 2). However, in order to reconstruct a detailed scheme of karyotype evolution within the genus *Lasiochernes*, data is needed from more species.

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