

# The first karyotype study in palpigrades, a primitive order of arachnids (Arachnida: Palpigradi)

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**Abstract** Chromosomes of palpigrades (Arachnida: Palpigradi), a rare arachnid order with numerous primitive characters, were studied for the first time. We analysed two species of the genus *Eukoenenia*, namely *E. spelaea* and *E. mirabilis*. Their karyotypes are uniform, consisting of a low number of tiny chromosomes that decrease gradually in size. Study of the palpigrade karyotype did not reveal morphologically differentiated sex chromosomes. Analysis of *E. spelaea* showed that constitutive heterochromatin is scarce, GC-rich, and restricted mostly to presumed centromeric regions. Meiosis is remarkable for the presence of a short diffuse stage and prominent nucleolar activity. During prophase I, nuclei contain a large nucleolus. Prominent knob at the end of one bivalent formed by constitutive heterochromatin is associated to the nucleolus

by an adjacent NOR. Presence of a nucleolus-like body at male prophase II suggests activity of NOR also during beginning of the second meiotic division. The data suggest acrocentric morphology of palpigrade chromosomes. Palpigrades do not display holocentric chromosomes which appear to be apomorphic features of a number of arachnid groups. These are: acariform mites, buthid scorpions, and spiders of the superfamily Dysderoidea. Therefore, cytogenetic data do not support a close relationship of palpigrades and acariform mites as suggested previously.

**Keywords** Arachnida · Holocentric chromosomes · Karyotype · Kinetochores · Palpigradi · NOR · Nucleolus · Phylogeny

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## Introduction

Over last decades, knowledge on cytogenetics of arthropods has increased. Nevertheless, karyotypes of some groups are still poorly understood or not available at all. This is especially true for some orders of Arachnida, one of the major and oldest classes of arthropods, which exhibit enormous species diversity. Approximately 97,000 species of arachnids, classified usually into 13 orders, have been described (Harvey 2002). However, information on arachnid karyology is still fragmentary. While more studies have been done in some species-rich orders, namely spiders (Araneae) (Král et al. 2006), mite orders Acariformes and Parasitiformes (Oliver 1977; Norton et al. 1993), harvestmen (Opiliones) (Tsurusaki 2007), scorpions (Scorpiones) (Shanahan 1989a, b), and pseudoscorpions (Pseudoscorpiones) (Št'áhlavský et al. 2006), karyotypes of the other orders are almost or completely unknown. Therefore it is still impossible to propose even a basic scheme for

karyotype evolution in the arachnids (Král 1994b). However, the data available indicate an unusual diversity of genome size as well as karyotype in the arachnid lineage. Despite the limited number of orders studied, the haploid genome size ( $C$  value) ranges greatly from 0.08 pg found in the smallest arthropod genome of acariform mite *Tetranychus urticae* (Gregory 2007) to 7.50 pg of parasitiform mite *Boophilus microplus* (Ullmann et al. 2005).

The extraordinary range of diploid chromosome numbers in arachnids reflects heterogeneity of their genome sizes. Reported  $2n$  varies from 4 (acariform mite *Harpyrhynchus brevis*) (Oliver and Nelson 1967) to 175 (scorpion *Urodacus novaehollandiae*) (Shanahan 1989b). Chromosomes of acariform mites (Oliver 1977), spiders of the superfamily Dysderoidea (Král et al. 2006), and scorpions of the family Buthidae (Shanahan 1989a) are holocentric (holokinetic); other studied groups possess normal chromosomes with localised centromere (i.e. monocentric chromosomes). Similarly to insects, arachnids display a considerable diversity of reproductive mechanisms as well as sex chromosome systems (Norton et al. 1993; Král 1994b). Furthermore, the heterogametic sex of some scorpions, acariform mites, pseudoscorpions, and spiders exhibits achiasmatic meiosis (Št'áhlavský and Král 2004).

Our study presents the first karyotype analysis of palpigrades (Palpigradi), the rare arachnid order with numerous primitive characters, namely three-segmented chelicerae, segmented prosoma and opisthosoma, leg-like pedipalps, and a multisegmented flagellum. In these respects, Palpigradi support the conception of a hypothetical ancestral arachnid (Savory 1971). In spite of this, the phylogenetic position of palpigrades within Arachnida is still controversial. Some authors considered palpigrades as sister group to the tetrapulmonate lineage of arachnids based on morphology (Shultz 1990) as well as on combination of morphological and molecular data of chelicerates (Wheeler and Hayashi 1998). The tetrapulmonate clade comprises four recent arachnid orders, namely Araneae, Amblypygi, Uropygi, Schizomida, and an extinct order Trigonotarbida (Selden et al. 1991). On the contrary, van der Hammen (1989) related palpigrades to the acariform mites. The latest combined analysis of arachnid phylogeny, which includes also molecular markers of palpigrades, brought them into a clade that involves orders Ricinulei, Trigonotarbida, Acari, and tetrapulmonates (Giribet et al. 2002). Hence, more data are required to determine the phylogenetic position of palpigrades.

To date, nearly 80 species of palpigrades, classified into 6 genera and 2 families, have been described. They live primarily in soils and terrestrial interstitial habitats of the tropics. European representatives inhabit mostly subterranean environments. The biology of palpigrades is almost

unknown because it is difficult to find living specimens and to keep them alive for a long time (Condé 1996).

In this study we describe karyotypes of two palpigrade species. *Eukoenia spelaea* (Peyerimhoff 1902) is documented from caves of the Alps, Western Carpathians and Dinaric Mountains. The species is rather polymorphic, with unclear subspecies taxonomy (Kováč 1999; Kováč et al. 2002). A unique abundant population from the Ardovská Cave (Slovak Karst, Slovakia) enabled us to obtain a sufficient number of specimens to analyse karyotype and course of meiosis. Some specimens were used for another study, which dealt with phylogeny of telomeric repeats in arthropods (Vítková et al. 2005). Furthermore, basic karyotype data were obtained for Mediterranean soil species *E. mirabilis* (Grassi & Calandruccio 1885) that has been accidentally transported with humans to Australia, Chile, South Africa, and Madagascar (Harvey et al. 2006).

## Material and methods

### Specimens

Specimens of *E. spelaea* were obtained from cave sediment of the Ardovská Cave (Slovak Karst, Slovakia; grid references 48°31'20'' N, 20°25'23'' E). Collected specimens were kept in cooled plastic vials with moistened plaster of Paris charcoal bottom (Kováč et al. 2002). A total of 60 specimens have been analysed, a material collected during seven trips: September 2000, August 2001, October and November 2002, February 2003, June and July 2004. Voucher specimens are deposited in collection of L.K., who also determined them. Twenty specimens of *E. mirabilis* were collected under stones in open forest in the neighbourhood of Wungong Dam (20 km SE of Perth) at western Australia (grid references 32°11'41'' S, 116°03'33'' E) during August 2003 (see Harvey et al. 2006 for details). Voucher specimens are housed in collection of the Western Australian Museum (Perth, Australia) (Harvey et al. 2006).

Determination of sex and developmental stage of palpigrades is possible only by morphology study of complete specimens on permanent preparations in light microscope. It was impossible to obtain these data before experiments because we used complete specimens for preparation of chromosomes. However, the presence of oocytes on preparations from female individuals allowed determining their sex and distinguishing them from the males. This approach failed only in young nymphs because they did not contain meiotic cells. We found some males and females with meiotic cells (i.e. with uncompleted meiosis) over the whole year. Male specimens were generally more suitable for karyotype study. In contrast to females, they provided

higher number of dividing cells and a complete sequence of meiotic stages. Concerning meiosis, only prophase I nuclei could be examined in females. Later stages of meiosis were either not available or invisible due to accumulation of yolk in oocytes.

### Chromosome preparations

Chromosomes were obtained from the whole content of opisthosoma. Majority of observed dividing cells came apparently from paired tubular gonads that are located along the axis of opisthosoma (Millot 1949). Dividing cells were only rarely found in prosoma.

Preparations were made using a modification of the spreading technique described by Pekár and Král (2001). Treatment by hypotonic solution (0.075 M KCl) was omitted because such exposure led to decay of palpigrade tissues. Palpigrades were killed by dipping into freshly prepared Carnoy fixative (ethanol, chloroform, and acetic acid, 6:3:1). Prosoma was removed by a pair of fine tweezers and opisthosoma fixed for 35 min in three changes of the fixative with increasing time of each fixation step (5, 10, and 20 min respectively). At the end of fixation, the opisthosoma was halved transversely with the fine tweezers. Then half of the opisthosoma was transferred into a drop of 60% acetic acid on a clean slide. The fixed tissues were pressed out from the opisthosoma with the pair of fine tweezers and then quickly shredded as much as possible with a pair of fine tungsten needles. Finally, the slide was placed on a warm histological plate (surface temperature of 40°C) and the drop of dispersed tissue was allowed to evaporate while keeping it moving constantly using the fine tungsten needle. The slides were air-dried at room temperature overnight, and stained with 5% Giemsa solution (Merck) in Sörensen phosphate buffer (pH = 6.8) for 60 min.

Preparations were inspected in a Zeiss Jenaval microscope and selected figures were photographed on Kodak Technical Pan film. In *E. spelaea*, chromosomes of 10 mitotic metaphase plates from males were measured and evaluated. Relative chromosome lengths were calculated as a percentage of total chromosome length of the diploid set. In *E. mirabilis*, we did not dispose a sufficient number of metaphases to calculate relative chromosome lengths.

In some cases, methanol/acetic acid (3:1) mixture was used instead of Carnoy fixative; fixed tissues were dispersed by 45% acetic acid only. Such preparations were convenient for induction of chromosome bandings and visualisation of nucleolus organizer regions (NORs) by silver nitrate. C-banding was that of Sumner (1972), with some modifications. Briefly, slides were aged in oven at 60°C for 60 min, incubated in 0.2 N HCl for 45 min, and

rinsed with distilled water. After that, they were dried and immersed into saturated Ba(OH)<sub>2</sub> at 50°C for 3–5 min. Then slides were rinsed with distilled water, dried and incubated at 2xSSC (pH 7, 60°C) for 75 min. After rinsing, preparations were dried overnight and stained with 5% Giemsa in Sörensen phosphate buffer (pH 6.8) for 2 h.

For fluorescent banding, preparations were stained with chromomycine A<sub>3</sub>/methyl green or methyl green/DAPI according to Sola et al. (1992), with some modifications. All slides were preincubated in McIlvaine buffer (pH 7) supplemented by 10 mM MgCl<sub>2</sub> for 10 min. For chromomycine staining, 150 µl of fluorochrome (0.5 mg/ml in McIlvaine buffer) was placed on a slide and incubated under coverslip for 15 min in dark. Afterwards, preparations were rinsed briefly in the same buffer and immersed into HEPES/NaCl buffer (pH 7) containing methyl green at a concentration of 0.12 mg/ml. Finally, the slides were briefly rinsed in HEPES/NaCl buffer. Before DAPI staining, preparations were treated by solution of McIlvaine buffer (pH 7) containing methyl green at concentration of 0.35 mg/ml for 15 min. After dripping, slides were immersed into solution of DAPI (0.5 mg/ml in McIlvaine buffer) for 15 min. Fluorescent preparations were mounted in antifade (2.5% *n*-propylgalate in glycerol) and observed.

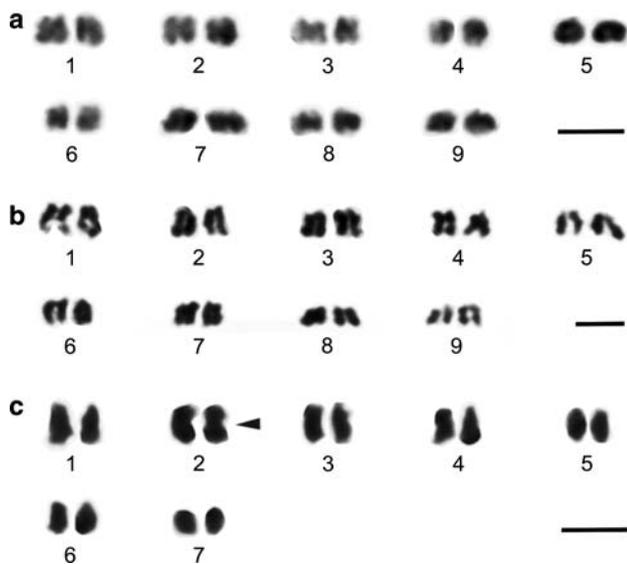
Preparations stained by fluorochromes were used later for detection of NORs. After washing off fluorochromes, staining of NORs by AgNO<sub>3</sub> was experienced by the 1-step method with colloidal developer (Howell and Black 1980).

The preparations with induced bands or visualised NORs were inspected in an Olympus AX 70 Provis microscope. Black-and-white images of chromosome plates were recorded with a CCD camera (SenSys, Photometrics Ltd., USA).

### Results

Karyotype of both species of the genus *Eukoenenia* is composed of tiny chromosomes that gradually decrease in size (Fig. 1a–c). Mitotic metaphase of *E. spelaea* consisted of 18 chromosomes (Fig. 1a, b). Relative lengths of metaphase chromosomes ranged from 7.1 to 4.1%. Chromosome complement of *E. mirabilis* contained 14 chromosomes (Fig. 1c).

Mitotic prophase of *E. spelaea* exhibited one or two prominent nucleoli. Each nucleolus was associated with one or several chromosomes. One chromosome associated with nucleolus usually showed a subterminal secondary constriction (Fig. 2b). Prometaphase and metaphase chromosomes showed neither primary nor secondary constriction (Fig. 1a, b). In some metaphase plates, chromosomes exhibited widely separated chromatids, which remained aligned usually by one end (Fig. 1b). Similarly,



**Fig. 1** Karyotypes of the genus *Eukoenenia* (based on mitotic plates). *E. spelaea* (a) male and (b) female metaphase. Note sister chromatids are associated each other by one end (b). (c) *E. mirabilis*, male metaphase. Arrowhead – constriction in the middle of chromosome pair No. 2. Giemsa staining, bar = 5  $\mu\text{m}$

metaphase chromosomes of *E. mirabilis* contained none constriction except pair No. 2 that showed indistinct constriction in the middle of chromosomes (Fig. 1c). Kinetic behaviour of palpi-grade chromosomes during mitotic anaphase remains unknown because we did not found unambiguous anaphase cells at our material.

The course of palpi-grade meiosis was studied on preparations of *E. spelaea* stained by Giemsa or fluorochromes. In contrast to males, females gave only plates of prophase I. Pachytene bivalents exhibited a well-developed pattern of chromomeres (Figs. 2c, 3d). Following pachytene, nuclei of both sexes entered a short diffuse stage with bivalents showing considerable despiralisation (Fig. 2d). Diplotene and diakinesis nuclei of both sexes contained nine bivalents; each bivalent showed one terminal or interstitial chiasma (Fig. 2e, f). At male metaphase I, bivalents formed distinct metaphase plates (Fig. 2g); all chiasmata were strongly terminalised (Fig. 2g, h). Meiotic chromosomes were arranged parallel to the spindle axis during metaphase and anaphase of both meiotic divisions, i.e. chromosomes were faced to a pole by one end (Fig. 2g, i, k). We did not identify interkinetic nuclei.

Nuclei of both sexes displayed a prominent nucleolus during prophase of the first meiotic division. One bivalent showed a subterminal NOR, which was associated with the nucleolus during pachytene. The end of the bivalent adjacent to NOR was formed by knob (Fig. 2c). Size of the nucleolus enlarged at the diffuse stage (Fig. 2d). During diplotene and diakinesis, the nucleolus usually somewhat lessened (Fig. 2e, f) being associated with the whole

NOR-bearing bivalent (Fig. 2f) whose terminal parts showed frequently a knob similar to that found at pachytene (Fig. 2e). Female nucleoli were bigger than male nucleoli during late prophase I. Nucleoli disappeared during the shift from prophase to metaphase I. Plates of metaphase I contained often one or two grains formed apparently by the residual of nucleolus (Fig. 2g, h). In some plates, these grains remained to be still associated with terminal parts of NOR-bearing bivalent (Fig. 2h). Interestingly, one or both sister prophase II exhibited a nucleolus-like body that was associated with one or several chromosomes (Fig. 2j). These bodies possessed a similar morphology to nucleoli of the first meiotic division however with a lower affinity to the Giemsa dye.

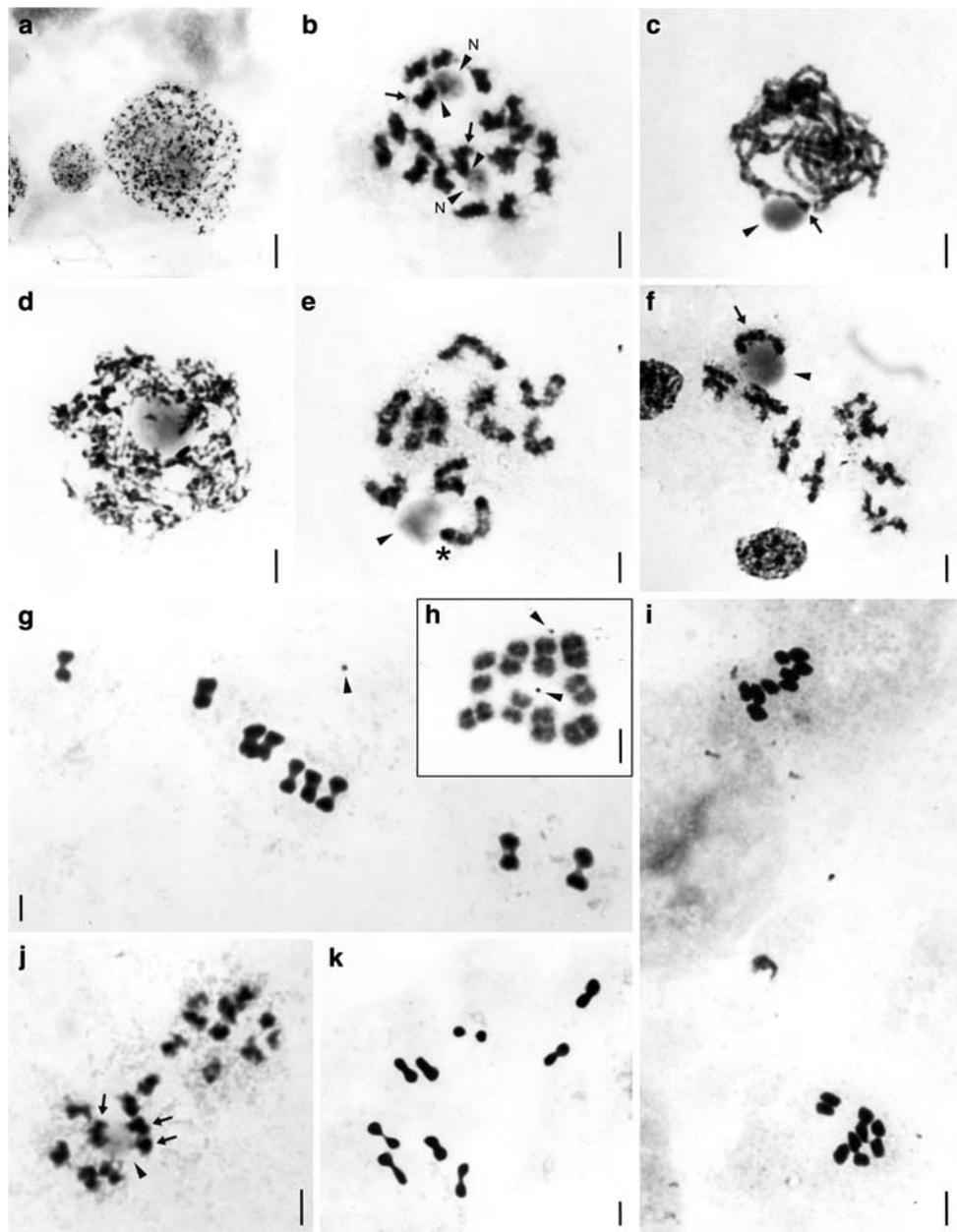
Apart from standard interphase nuclei, we observed infrequently also giant interphase nuclei on preparations of both sexes (Fig. 2a). Their large size indicated an endopolyploid origin.

Sex chromosomes were morphologically not differentiated. We were not able to distinguish them also according to their specific behaviour during the meiotic division.

Distribution of constitutive heterochromatin in the karyotype of *E. spelaea* was analysed by C-banding. Mitotic metaphases did not exhibit C-bands. C-banding pattern was induced on mitotic prophase I and some meiotic stages only, namely pachytene (Fig. 3a), metaphase I (Fig. 3b), and anaphase I. There was one prominent band associated with the nucleolus at the end of one pachytene bivalent (Fig. 3a). Some pachytene bivalents exhibited also a few tiny C-bands at the terminal and/or intercalary position (Fig. 3a). During metaphase and anaphase I, C-bands were localised mostly on chromosome termini faced to poles (Fig. 3b). To detect subclasses of heterochromatin, we treated some unstained preparations with fluorochromes possessing affinities for AT (DAPI) or GC base pairs (chromomycine A<sub>3</sub>), respectively. Sequential staining of pachytene bivalents with chromomycine A<sub>3</sub> and AgNO<sub>3</sub> demonstrated that distal part of bivalent associated with nucleolus (Fig. 3d) is GC-rich (Fig. 3f). We did not detect any other signal on chromosomes of *E. spelaea* after application of fluorochromes. Staining of interphase nuclei by fluorochromes revealed two GC-rich blocks on the periphery of nucleolus (Fig. 3g). Association of these blocks with periphery of the nucleolus suggests that they correspond to the GC-rich block of NOR-bearing bivalent.

The Ag-NOR staining method serves to demonstrate nucleoli in interphase nuclei as well as NORs on the mitotic chromosomes (Howell and Black 1980) which have been active in the preceding interphase (Miller et al. 1976). Despite association of prophase nucleoli with several chromosomes, NORs were visualized on one chromosome pair only, namely in terminal position (Fig. 3c). Our

**Fig. 2** *Eukoenenia spelaea*; interphase, mitotic and meiotic divisions. Unless otherwise indicated, based on male plates. (a) Two interphase nuclei. Note size difference between standard (left) and endopolyploid nucleus (right). (b) Late mitotic prophase. Arrowheads point to the subterminal secondary constrictions of chromosomes (arrows) associated with nucleolus (N). (c) Pachytene. Terminal knob of one bivalent (arrow) is associated with a large nucleolus (arrowhead). (d) Diffuse stage. (e) Late diplotene. Large distal knob of one bivalent (asterisk) is associated with the nucleolus (arrowhead). (f) Female diakinesis. Note bivalent associated by both ends (arrow) with the nucleolus (arrowhead). (g, h) Metaphase I with nine bivalents: lateral (g) and polar view (h). Note remnants of nucleolus (arrowhead) that remain to be associated with one bivalent at some plates (h). (i) Anaphase I. (j) Two sister prophase II nuclei. One sister plate contains nucleolus-like body (arrowhead) that is associated with several chromosomes (arrows). (k) Metaphase II. Giemsa staining, bar = 10  $\mu$ m (a) or 5  $\mu$ m (b–k)



attempts to visualize NORs in mitotic metaphases failed. Treatment of meiotic chromosomes by  $\text{AgNO}_3$  induced one or two distinct dots on chromosome ends faced to pole during metaphase and anaphase I (Fig. 3e, h).

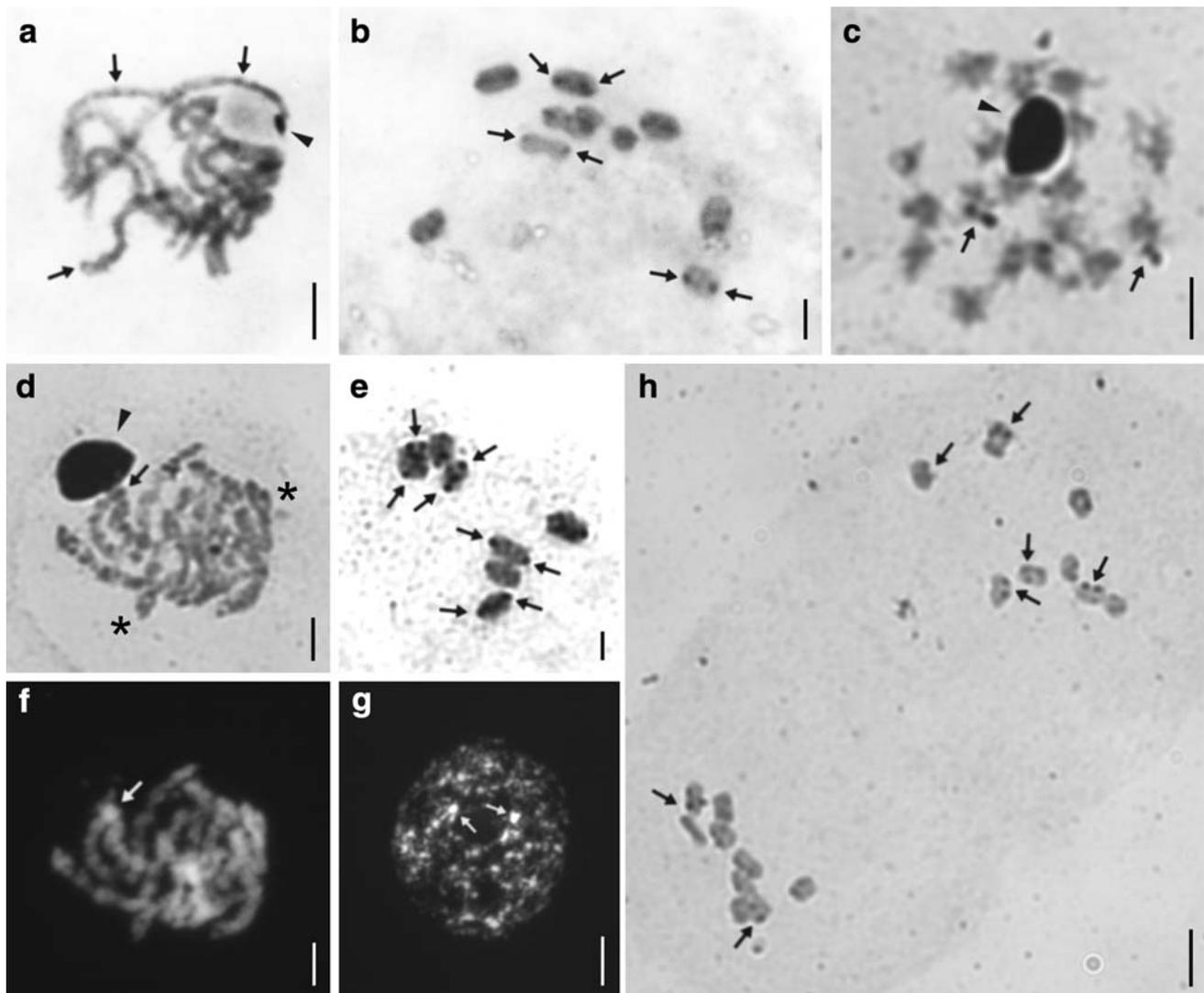
## Discussion

Our results demonstrate that the karyotype of studied paligrades is morphologically uniform, formed by a small number of tiny chromosomes that decrease gradually in size. However, difference in diploid chromosome number of studied species, *E. spelaea* and *E. mirabilis*, indicates

that karyotype diversity of the genus *Eukoenenia* could be sufficient to solve some taxonomic problems of this morphologically uniform genus.

Chromosomes of *E. spelaea* exhibit no visible primary constrictions. One chromosome pair shows a subterminal secondary constriction during mitotic prophase. This constriction is associated with a prominent nucleolus. We assume that constriction in the middle of second chromosome pair of *E. mirabilis* is also secondary because we did not observe any biarmed chromosome in meiosis of this species.

Chromosomes of *E. spelaea* possess a low amount of constitutive heterochromatin, which occupies mostly terminal location. One chromosome pair shows a large



**Fig. 3** *Eukoenia spelaea*, differential staining of male chromosomes. (a) C-banded pachytene. Arrowhead indicates a large block of constitutive heterochromatin associated with the nucleolus. Some bivalents contain tiny intercalary or terminal blocks of heterochromatin (arrows). (b) C-banded metaphase I. Note blocks of heterochromatin in terminal parts of bivalents directed to the pole (arrows). (c) Mitotic prophase, silver staining. Two chromosomes bear terminal NOR (arrow). Arrowhead – nucleolus associated with several chromosomes. (d) Transition from zygotene to pachytene, silver staining. Nucleolus (arrowhead) is associated with terminal part of one bivalent (arrow). Terminal parts of some bivalents are still formed by unpaired

chromosomes (asterisks). (e) Incomplete metaphase I (7 bivalents), silver staining. Note presumable kinetochores at terminal parts of bivalents faced to the poles (arrows). (f) The same transition from zygotene to pachytene as seen at Fig. 3d, chromomycine staining. Terminal NOR and adjacent constitutive heterochromatin exhibit bright fluorescence (arrow). (g) Interphase nucleus, chromomycine staining. GC-negative nucleolus forms circular dark area in the middle of the nucleus. Note two blocks of GC-rich heterochromatin on the periphery of the nucleolus (arrows). (h) Anaphase I, silver staining. Note presumable kinetochores at one end of chromosomes (arrows). Bar = 5 $\mu$ m

terminal block of heterochromatin in pachytene nuclei. An adjacent NOR is associated with the prominent nucleolus that disappears during the shift from prophase to metaphase I. Sequential staining by chromomycine A<sub>3</sub> and silver nitrate revealed that both the NOR and the adjacent block of constitutive heterochromatin are GC-rich. NOR-bearing bivalent is apparently homologous to the pair of mitotic chromosomes containing the subterminal secondary constriction.

An unusual character of palpi-grade meiosis is the presence of nucleolus-like bodies at prophase II. In general, meiotic nucleoli disappear at the latest from metaphase I (Sumner 2003). Persistence of nucleoli during following meiotic stages including second meiotic division was found only in some invertebrates, bugs (Heteroptera) (Cattani and Papeschi 2004) and crickets (Grylloidea; Orthoptera), for example (Satya-Prakash and Pathak 1984). In contrast to these insects, nucleolus-like bodies of palpi-grades arise de

novo during prophase II. Unfortunately, prophase II plates were rarely observed in our material, which excluded demonstration of NOR activity by silver staining.

Beside the prominent nucleolar activity, meiotic division of palpigrades is characterised also by transient but a nearly complete despiralisation of bivalents between pachytene and diplotene. Based on the bivalent morphology and timing in prophase I, we interpret these plates as a diffuse stage. Till now, the diffuse stage was found in various plants (Klášťerská 1977) and animals including heteropteran insects (e.g., Cattani and Papeschi 2004; Franco et al. 2006) and also in arachnids, such as some acariform mites, ticks, harvestmen, pseudoscorpions, and spiders (reviewed by Král et al. 2006; Št'áhlavský et al. 2006). We suggest that the expansion of palpigrade nucleolus during the diffuse stage reflects an increase of the transcriptional activity. The presence of the diffuse stage and prominent activity of NORs during both meiotic divisions enable spermatocytes to produce a large amount of stable RNA transcripts required for completion of spermiogenesis (Severi-Aguiar et al. 2006). Frequently observed mitotic prophase with large nucleoli indicate a considerable activity of NORs also during spermatogonial prophase of palpigrades. We suppose that the enhanced production of RNA transcripts during the male meiosis of palpigrades is necessary to ensure complex changes of sex cell morphology that are characteristic for the subsequent spermiogenesis of these arachnids (Alberti 1979).

Enhanced metabolic activity is typical also for endopolyploid nuclei of somatic cells (Macgregor 1993) that we observed on preparations. Spreading technique used for preparation of chromosomes disintegrates tissues to separate cells so that we are not able to determine a source for these cells. We hypothesize endopolyploid cells came from palpigrade tissue(s) exhibiting a considerable metabolic activity, for example gonads, digestive or spinning glands. Among arachnids, endopolyploid nuclei were reported only in some spiders, namely in somatic cells of testes (Sokolov 1967) and spinning glands (Gregory and Shorthouse 2003).

Analysis of karyotype and chromosome behaviour during meiosis has not revealed the presence of sex chromosomes. This suggests that sex chromosomes of palpigrades are morphologically undifferentiated (i.e. homomorphic) or not differentiated at all. Distribution of sex chromosomes within subphyllum Chelicerata indicates that the absence of differentiated sex chromosomes found in palpigrades could be an ancestral state of the class Arachnida. Sex chromosomes have not been demonstrated in two basal chelicerate clades, namely merostomates (Iwasaki et al. 1988) and scorpions (Guénin 1957, 1961). Beside these groups, differentiated sex chromosomes have not been found also in acariform mites, except for some families of the suborder Acaridida (Norton et al. 1993) that

was recognised, however, to be derived acariform lineage (Norton 1998).

To solve phylogenetic position of palpigrades, it is of special importance to determine kinetic behaviour of their chromosomes. Some authors regarded palpigrades as a sister group of the tetrapulmonate clade (Shultz 1990; Wheeler and Hayashi 1998), some other related them to acariform mites (van der Hammen 1989). Karyotypes of tetrapulmonate orders, namely whip spiders (Amblypygi), whip scorpions (Uropygi), schizomids (Schizomida) (Král J, Št'áhlavský F, Musilová J and Sember A unpublished), and most spiders (Araneae) (Král et al. 2006) are composed by monocentric chromosomes. In contrast to this, karyotypes of acariform mites consist of a low number of tiny holocentric chromosomes (Oliver 1977). Holocentric chromosomes represent a derived type of chromosomes. They are polyphyletic, originating independently several times during evolution of eukaryotes (Král 1994a; Maddox et al. 2004). Most probably these chromosomes evolved from monocentric chromosomes by a gradual expansion of kinetic activity over large area of chromosome surface (Král 1994a; Nagaki et al. 2005). Regarding the apomorphic state of holocentric chromosomes, they represent a potential character for cladistic analysis. Synapomorphic presence of holocentric chromosomes in the spider superfamily Dysderoidea (Král et al. 2006) or insect orders Lepidoptera and Trichoptera forming a common clade, the Amphiesmenoptera (Marec and Novák 1998), may serve as the examples.

Alignment of sister chromatids at one terminus during mitotic metaphase indicates acrocentric morphology of palpigrade chromosomes. However, telokinetic behaviour of meiotic chromosomes found in palpigrades is typical not only for acrocentric but also for primitive holocentric chromosomes, which remain functionally monocentric during meiosis with spindle fibers attached to the telomere. Strictly speaking, primitive holokinetic chromosomes are potentially dicentric during meiosis because both chromosome termini can exhibit kinetic activity (Pérez et al. 1997). In order to obtain more data about kinetic behaviour of palpigrade chromosomes, we analysed their meiosis by Ag-NOR staining and C-banding. Apart from NORs, silver staining also shows chromosome cores, heterochromatin, kinetochores, and synaptonemal complexes of some organisms (Sumner 1990). In palpigrades, silver staining revealed argentophilic dots at chromosome ends faced to poles during the first meiotic division. Based on morphology and position, we consider these structures to be kinetochores. In contrast to meiosis, kinetochores of mitotic chromosomes were not stained. Absence of affinity to silver was observed also in mitotic kinetochores of mammals (Sudman and Greenbaum 1988). Different silver staining affinity of mitotic kinetochores suggests fundamental structural differences between mitotic and meiotic

kinetochores (Sudman and Greenbaum 1988). Kinetochores nature of argentophilic material of palpigrades is corroborated by the same location of presumable kinetochores and most C-bands. Asymmetric distribution of palpigrade heterochromatin that is located mostly at areas of presumable kinetochores is not characteristic for holocentric chromosomes (Král 1994a) but it is common in acrocentric chromosomes. Therefore, our data altogether suggest acrocentric morphology, i.e. monocentric structure of palpigrade chromosomes. Providing acrocentric morphology of chromosomes in both studied species, the karyotype of *E. mirabilis* could be derived from karyotype of *E. spelaea* by series of two tandem fusions.

The monocentric structure of palpigrade chromosomes does not support a close relationship between palpigrades and acariform mites, because the latter possess holocentric chromosomes. On the contrary, our preliminary data indicate possible relationship of palpigrades to so-called pedipalps, i.e. tetrapulmonate branch formed by orders Amblypygi, Schizomida, and Uropygi. Besides monocentric chromosomes, karyotypes of the most pedipalps do not contain morphologically differentiated sex chromosomes (Král J, Št'áhlavský F, Musilová J, Sember A, unpublished). However, to gain insights into palpigrade relationships, detailed data on structure and evolution of karyotypes in pedipalps as well as other arachnid groups are necessary.

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## References

- Alberti G (1979) Zur Feinstruktur der Spermie und Spermiocyto-genese von *Prokoenenia wheeleri* (Rucker, 1901) (Palpigradi, Arachnida). *Zoomorphologie* 94:111–120
- Cattani MV, Papeschi AG (2004) Nucleolus organizing regions and semi-persistent nucleolus during meiosis in *Spartocera fusca* (Thunberg) (Coreidae, Heteroptera). *Hereditas* 140:105–111
- Condé B (1996) Les Palpigrades, 1885–1995: acquisitions et lacunes. *Rev Suisse Zool* hors série 1:87–106
- Franco MJ, Bressa MJ, Papeschi AG (2006) Karyotype and male meiosis in *Spartocera batatas* and meiotic behaviour of multiple sex chromosomes in Coreidae (Heteroptera). *Eur J Entomol* 103:9–16
- Giribet G, Edgecombe GD, Wheeler WC, Babbitt C (2002) Phylogeny and systematic position of Opiliones: a combined analysis of chelicerate relationships using morphological and molecular data. *Cladistics* 18:5–70
- Gregory TR (2007) Animal genome size database. <http://www.genomesize.com>. Cited 15 March 2007
- Gregory TR, Shorthouse DP (2003) Genome sizes of spiders. *J Heredity* 94:285–290
- Guénin HA (1957) Contribution à la connaissance cytologique des Scorpions: les chromosomes de *Pandinus imperator* Koch. *Rev Suisse Zool* 64:349–353
- Guénin HA (1961) Contribution à la connaissance cytologique des Scorpions: les chromosomes de *Buthus occitanus* Amor. *Vie et Milieu* 12:89–96
- Howell WM, Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* 36:1014
- Harvey MS (2002) The neglected cousins: what do we know about the smaller arachnid orders? *J Arachnol* 30:357–372
- Harvey MS, Št'áhlavský F, Theron PD (2006) The distribution of *Eukoenenia mirabilis* (Palpigradi: Eukoeneiidae): a widespread tramp. *Rec West Austr Mus* 23:199–203
- Iwasaki Y, Iwami T, Sekiguchi K (1988) Karyology. In: Sekiguchi K (ed) *Biology of horseshoe crabs*. Science House Co., Ltd., Tokyo
- Klásterská I (1977) The concept of the prophase of meiosis. *Hereditas* 86:205–210
- Kováč L (1999) *Eukoenenia spelaea* (Peyerimhoff, 1902)—a cave dwelling palpigrade species (Arachnida, Palpigradi) from the Slovak Karst. In: Tajovský K, Pižl V (eds) *Soil zoology in central Europe*. Proceedings of the 5th central European workshop on soil zoology, August 1999. Institute of Soil Biology AS CR, České Budějovice, Czech Republic, pp 157–160
- Kováč L, Mock A, L'uptáčík P, Palacios-Vargas JG (2002) Distribution of *Eukoenenia spelaea* (Peyerimhoff, 1902) (Arachnida, Palpigradida) in the Western Carpathians with remarks on its biology and behaviour. In: Tajovský K, Balík V, Pižl V (eds) *Studies on soil fauna in central Europe*. Institute of Soil Biology AS CR, České Budějovice, Czech Republic, pp 93–99
- Král J (1994a) Holokinetic (holocentric) chromosomes. *Biologické listy* 59:191–217 (at Czech, with English summary)
- Král J (1994b) Review of arachnid cytogenetics. *Biologické listy* 59:282–306 (at Czech, with English summary)
- Král J, Musilová J, Št'áhlavský F, Řezáč M, Akan Z, Edwards RL, Coyle FA, Ribera Almerje C (2006) Evolution of the karyotype and sex chromosome systems in basal clades of araneomorph spiders (Araneae: Araneomorphae). *Chromosome Res* 14:859–880
- Macgregor HC (1993) *An introduction to animal cytogenetics*. Chapman & Hall, London
- Maddox PS, Oegema K, Desai A, Cheeseman IM (2004) “Holo” er than thou: chromosome segregation and kinetochores function in *C. elegans*. *Chromosome Res* 12:641–653
- Marec F, Novák K (1998) Absence of sex chromatin corresponds with a sex-chromosome univalent in females of Trichoptera. *Eur J Entomol* 95:197–209
- Miller DA, Dew V, Tantravahi R, Miller O (1976) Suppression of human nucleolar organizer activity in mouse human somatic hybrid cells. *Exp Cell Res* 101:235–243
- Millot J (1949) Ordre des palpigrades. In: Grassé PP (ed) *Traité de Zoologie, Anatomie, Systématique, Biologie*. Tome VI.

- Onychophores, Tardigrades, Arthropodes – Trilobitomorphae, Chélicérates. Masson et Cie Éditeurs, Paris
- Nagaki K, Kashihara K, Murata M (2005) Visualization of diffuse centromere with centromere-specific histone H3 in the holocentric plant *Luzula nivea*. *Plant Cell* 17:1886–1893
- Norton RA (1998) Morphological evidence for the evolutionary origin of Astigmata (Acari: Acariformes). *Exp Appl Acarol* 22:559–594
- Norton RA, Kethley JB, Johnston DE, OConnor BM (1993) Phylogenetic perspectives on genetic systems and reproductive modes of mites. In: Wrensch D, Ebbert M (eds) *Evolution and diversity of sex ratio in insects and mites*. Chapman & Hall, London
- Oliver JH (1977) Cytogenetics of mites and ticks. *Ann Rev Ent* 22:407–429
- Oliver JH, Nelson BC (1967) Mite chromosomes: an exceptionally small number. *Nature* 214:809
- Pekár S, Král J (2001) A comparative study of the biology and karyotypes of two central European zodariid spiders (Araneae, Zodariidae). *J Arachnol* 29:345–353
- Pérez R, Panzera F, Page J, Suja JA, Rufas JS (1997) Meiotic behaviour of holocentric chromosomes: orientation and segregation of autosomes in *Triatoma infestans* (Heteroptera). *Chromosome Res* 5:47–56
- Satya-Prakash KL, Pathak S (1984) Silver staining pattern of male meiosis in the house cricket. *J Heredity* 75:319–320
- Savory TH (1971) *Evolution in the Arachnida*. Mellow Publishing Co. Ltd., Watford, Great Britain
- Selden PA, Shear WA, Bonamo PM (1991) A spider and other arachnids from the Devonian of New York, and reinterpretations of Devonian Araneae. *Palaeontology* 34:241–281
- Severi-Aguiar GDC, Laurenço LB, Bicudo HEMC, Azeredo-Oliveira MTV (2006) Meiosis aspects and nucleolar activity in *Triatoma vitticeps* (Triatominae, Heteroptera). *Genetica* 126:141–151
- Shanahan CM (1989a): Cytogenetics of Australian scorpions. I. Interchange polymorphism in the family Buthidae. *Genome* 32:882–889
- Shanahan CM (1989b) Cytogenetics of Australian scorpions. II. Chromosome polymorphism in species of *Urodacus* (family Scorpionidae). *Genome* 32:890–900
- Shultz JW (1990) Evolutionary morphology and phylogeny of Arachnida. *Cladistics* 6:1–38
- Sokolov II (1967) Endomitotic polyploidy in testicular epithelial cells of spiders (Araneina). II. *Cytology* 9:257–264 (at Russian, with English summary)
- Sola L, Rossi AR, Iaselli V, Rasch EM, Monaco PJ (1992) Cytogenetics of bisexual/unisexual species of *Poecilia*. II. Analysis of heterochromatin and nucleolar organizer regions in *Poecilia mexicana mexicana* by C-banding and DAPI, quinacrine, chromomycin A<sub>3</sub>, and silver staining. *Cytogenet Cell Genet* 60:229–235
- Sudman PD, Greenbaum IF (1988) Visualization of kinetochores in mammalian meiotic preparations and observations of argento-philic differences between mitotic and meiotic kinetochores. *Genome* 32:380–382
- Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res* 75:304–306
- Sumner AT (1990) *Chromosome banding*. Unwin Hyman, London
- Sumner AT (2003) *Chromosomes: organization and function*. Blackwell Science Ltd. & Blackwell Publishing Company, Malden, USA
- Št'áhlavský F, Král J (2004) Karyotype analysis and achiasmatic meiosis in pseudoscorpions of the family Chthoniidae (Arachnida: Pseudoscorpiones). *Hereditas* 52:1–12
- Št'áhlavský F, Král J, Harvey MS, Haddad CR (2006) A karyotype study on the pseudoscorpion families Geogarypidae, Garypiniidae and Olpiidae (Arachnida: Pseudoscorpiones). *Eur J Entomol* 103:277–289
- Tsurusaki N (2007) Cytogenetics. In: Pinto-da-Rocha R, Machado G, Giribet G (eds) *Harvestmen: the biology of Opiliones*. Harvard University Press, Cambridge, USA
- Ullmann AJ, Lima CMR, Guerrero FD, Piesman J, Black WC (2005) Genome size and organization in the blacklegged tick, *Ixodes scapularis* and the Southern cattle tick, *Boophilus microplus*. *Insect Mol Biol* 14:217–222
- van der Hammen L (1989) *An introduction to comparative arachnology*. SPB Acad. Publ., The Hague, Netherlands
- Vítková M, Král J, Traut W, Zrzavý J, Marec F (2005) The evolutionary origin of insect telomeric repeats, (TTAGG)<sub>n</sub>. *Chromosome Res* 13:145–156
- Wheeler WC, Hayashi CY (1998) The phylogeny of the extant chelicerate orders. *Cladistics* 14:173–192