Chromosome complement and meiosis of *Holmbergiana weyenberghii* (Opiliones: Sclerosomatidae: Gagrellinae) from Argentina

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Complemento cromosómico y meiosis de *Holmbergiana weyenberghii* (Opiliones: Sclerosomatidae: Gagrellinae) de Argentina

■ **RESUMEN.** Se analiza citogenéticamente, por primera vez, una especie de opilión proveniente de Argentina: *Holmbergiana weyenberghii* (Holmberg) (Eupnoi, Sclerosomatidae, Gagrellinae). Los machos tienen un complemento cromosómico compuesto por 18 cromosomas. En meiosis, hay nueve bivalentes homomórficos: uno mayor, cinco medianos y tres menores. El número cromosómico de *H. weyenberghii* se encuentra dentro del rango de números diploides de los Gagrellinae Thorell; esta subfamilia presenta los números cromosómicos más bajos de Sclerosomatidae.

PALABRAS CLAVE. Comportamiento meiótico. *Holmbergiana weyenberghii*. Opiliones. Gagrellinae.

■ **ABSTRACT.** The cytogenetical analysis of the harvestman *Holmbergiana weyenberghii* (Holmberg) (Eupnoi, Sclerosomatidae, Gagrellinae) from Argentina is reported for the first time. The complement of males is composed of 18 chromosomes. In meiosis there are nine homomorphic bivalents: one large, five medium-sized and three small. The chromosome number of *H. weyenberghii* is within the range of diploid numbers of the subfamily Gagrellinae Thorell, which shows the lowest chromosome numbers among the sclerosomatids.

KEY WORDS. Meiotic behavior. *Holmbergiana weyenberghii*. Opiliones. Gagrellinae.

INTRODUCTION

The Order Opiliones Sundevall, comprises four suborders (Cyphophthalmi Simon, Eupnoi Hansen & Sørensen, Dyspnoi Hansen & Sørensen, and Laniatores Thorell) with more than 6.000 taxonomically described species (Shultz & Regier, 2001; Giribet *et al.*, 2002; Hallan, 2009). The family Sclerosomatidae Simon, of the suborder Eupnoi, includes four subfamilies from the Old and New World: Gagrellinae Thorell, Leiobuninae Banks, Sclerosomatinae Simon, and Gyinae Silhavy (Acosta & Maury, 1998; Hallan, 2009). The former two subfamilies are represented in Argentina; Gagrellinae, with

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about 1,100 species distributed worldwide, has the largest number of taxonomically described species of the family, and among these only 13 occur in Argentina (Acosta & Maury, 1998; Hallan, 2009). *Holmbergiana weyenberghii* Holmberg is an Argentine species typical of the Mesopotamian region, which is also found in the Pampean region (Acosta & Maury, 1998).

The cytogenetic analysis of harvestmen has been performed in less than 1,5% of taxonomically described species. These showed a wide range of diploid numbers, from 10 in *Systenocentrus japonicus* Hirst, *Paraumbogrella pumilio* (Karsch), some chromosome races of *Gagrellula ferruginea* (Loman) (Eupnoi) and some populations of *Sabacon makinoi* Suzuki (Dyspnoi), to 92-109 in *Goniosoma spelaeum* (Mello-Leitão) (Laniatores) (Oliveira *et al.*, 2006; Tsurusaki, 2007). Most species have non-distinguishable sex chromosomes, the XX/XY system (female/male) was found in several species of Gagrellinae and Leiobuninae (Sclerosomatidae, Eupnoi) and in *Sabacon makinoi* (Sabaconidae, Dyspnoi), while the ZW/ZZ system (female/male) was only observed in *Mitopus morio* (Fabricius) and *Odiellus aspersus* (Karsch) (Phalangiidae, Eupnoi) (Tsurusaki, 1985; Tsurusaki & Cokendolpher, 1990; Tsurusaki, 2007). The subfamilies of Sclerosomatidae analysed were Gagrellinae (7 species), Leiobuninae (28 species) and Sclerosomatinae (3 species); these belong to the Old World, except for five species of Leiobuninae occurring in USA (Tsurusaki, 1985, 2006, 2007).

This is the first cytogenetic study of an Argentine opilionid species, *Holmbergiana weyenberghii* (Sclerosomatidae,Gagrellinae), with a description of some spermatogonial chromosome characteristics and meiotic development.

MATERIAL AND METHODS

The specimens of *Holmbergiana weyenberghii* were collected in Buenos Aires City (34° 36′ 30″ S - 58° 22′ 23″ W) (five males, March 1997) and in the Horco Molle Ecological Reserve in San Javier Hill, Province of Tucumán (26° 46′ 22″ S - 62° 21′ 13″ W) (one male, September 1997), Argentina.

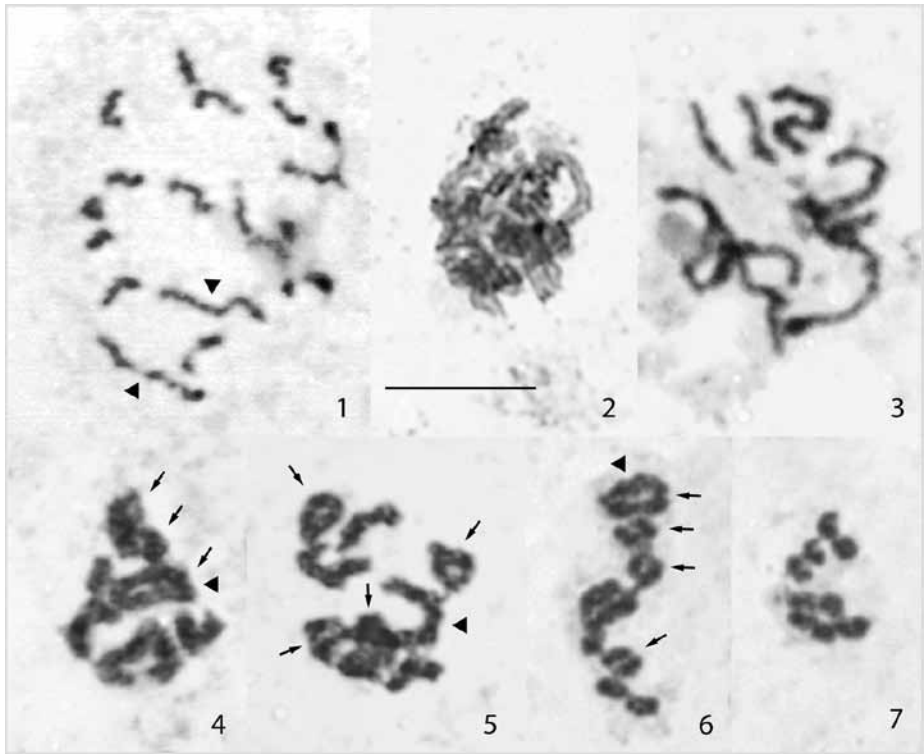
The specimens were transported alive to the laboratory and determined taxonomically by the late Dr. E. Maury (Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"). The harvestmen were anesthetised with ether prior to dissection of the testes, which were fixed in 3:1 (absolute ethylic alcohol: glacial acetic acid), preserved in 70% ethanol and stored in the refrigerator until use. Preparations were made by squashing the material in acetic hematoxylin.

RESULTS

The males of *Holmbergiana weyenberghii* showed a complement composed of 18 biarmed chromosomes. At spermatogonial late prophase the chromosomes show heterochromatic regions of different size in the telomeric regions and, to a lesser extent, in interstitial regions (Fig. 1). At early prophase I there is no positive heteropycnotic body (Figs. 2-3). Nine homomorphic bivalents are seen from diplotene onwards. One bivalent is conspicuously larger, five are medium-sized and decrease gradually in size and three are small (Figs. 4-6). The larger and the medium-sized bivalents may show two terminal chiasmata or a single terminal or sub-terminal chiasma; while the small bivalents only have one terminal chiasma (Figs. 4-6). All the chromosomes migrate synchronously at anaphase I, and at metaphase II nine chromosomes are seen (Fig. 7). No differences were observed between locations.

DISCUSSION

Thirty-eight species of Sclerosomatidae have been cytogenetically studied, and only seven of these belong to the subfamily Gagrellinae (Tsurusaki, 1985, 2006, 2007), which shows the lowest chromosome numbers. This subfamily has a diploid



Figs. 1-7. Mitosis and meiosis in *Holmbergiana weyenberghii* (2n=18, n=9); 1, Late spermatogonial prophase; 2, Pachytene; 3, Late pachytene; 4-5, Diakinesis; 6, Prometaphase I; 7, Metaphase II. The arrowheads point to the larger pair. The arrows point to the bivalents with two chiasmata. Scale bar = 10 μm.

chromosome number varying from 10 in *Systenocentrus japonicus*, *Paraumbogrella pumilio* and some chromosome races of *Gagrellula ferruginea*, to 22 in some populations of *Gagrellopsis nodulifera* Sato & Suzuki and other chromosome races of *Gagrellula ferruginea* (Tsurusaki *et al.*, 1991; Gorlov & Tsurusaki, 2000a; Tsurusaki, 2007). All available data on the Gagrellinae were exclusively obtained from material collected in Japan except for *Gagrella unicolor* (Roewer) (sub *Melanopa unicolor*) from India (Sharma & Dutta, 1959). Cytogenetic studies of South American harvestmen were performed in only eight species of the family Gonyleptidae Sundevall (Laniatores) from Brazil, which showed the highest chromosome numbers of the order (Oliveira *et al.*, 2006; Tsurusaki, 2007). The study of *Holmbergiana weyenberghii* represents the first contribution to the cytogenetic knowledge of Eupnoi in South America.

In Gagrellinae the sex determination system XY/XX (male/female) has been reported in four species: *Paraumbogrella pumilio, Psathyropus tenuipes* L. Koch (sub *Metagagrella tenuipes*), *Gagrellopsis nodulifera,* and *Gagrella unicolor* (Sharma and Dutta, 1959; Tsurusaki, 1982, 1993; Tsurusaki *et al.*, 1991). In *Gagrella unicolor* male meiosis the sex chromosomes are seen as one or two heteropycnotic bodies at early prophase I and as a heteromorphic pair from diplotene to metaphase I; furthermore they precede the migration of autosomes at both meiotic anaphases (Sharma and Dutta, 1959). In *Holmbergiana weyenberghii* neither of these features was seen; nevertheless, it is not possible to rule out the presence of sex chromosomes due to the lack of suitable metaphase plates to allow a detailed karyotypic analysis.

It is interesting to point out that the presence of two chiasmata in a variable number of bivalents seems to be frequent in Gagrellinae species, since besides *H. weyenberghii*, it has been reported in at least other four species, *Paraumbogrella pumilio, Psathyropus tenuipes, Gagrellopsis nodulifera,* and *Gagrella unicolor* (Sharma & Dutta, 1959; Tsurusaki, 1982, 1993; Tsurusaki *et al.,* 1991; Gorlov & Tsurusaki, 2000b).

The diploid number of *H. weyenberghii* (2n=18) falls within the range of the chromosome numbers of the subfamily; this diploid number is also found in *Gagrella unicolor* and *Psathyropus tenuipes*. In the latter species the chromosome number increases within and between populations due to the presence of a variable number of supernumerary chromosomes, from one to 18 B-chromosomes (Tsurusaki, 1993; Gorlov & Tsurusaki, 2000b). Other chromosome number variations in populations of Gagrellinae result from structural rearrangements giving rise to chromosome races as in *Gagrellula ferruginea* (2n=10-22) and *Gagrellopsis nodulifera* (2n=14-22). Several hybrid zones have been reported for the latter species in Japan, where neighbouring populations with different chromosome numbers come in contact and produce heterozygotic karyotypes with different chromosome numbers (Gorlov & Tsurusaki, 2000a; Tsurusaki, 2007).

Despite the limited cytogenetic studies on harvestmen, particular features have been reported for the order, such as different chromosomal sex-determination systems, B-chromosomes, polyploidy, chromosome races and hybrid zones in Old World species (Tsurusaki, 2007). Further research is needed to clarify the population cytogenetic features of New World species, representing only 21% of the chromosomally analysed species.

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