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CYTOGENETICS IN THREE SPECIES OF *POLYBETES* SIMON 1897 FROM ARGENTINA (ARANEAE, SPARASSIDAE) I. KARYOTYPE AND CHROMOSOME BANDING PATTERN

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ABSTRACT. Species of *Polybetes* are known exclusively from South America. Currently there are 13 described species, 9 occurring in Argentina. Cytogenetic studies in spiders are scarce; the cytogenetics of only about 1% of nearly 39,500 described species are known. Within the Sparassidae, 38 species out of 1,009 (< 4%) have been cytogenetically analyzed; the most frequent chromosome number is $2n = 43/46$ (male/female), $n = 20 + X_1X_2X_3$, present in almost half of the species studied. Female diploid chromosome number is only known for four species: *Heteropoda venatoria* (Linnaeus 1767) ($2n = 44$); *Pediana regina* (L. Koch 1875), *Isopeda* sp. and *Olios* sp. ($2n = 46$). Within the genus *Polybetes*, only *P. pythagoricus* (Holmberg 1875) had been previuosly cytogenetically analyzed. In the present work, the karyotype, heterochromatin content and distribution, and silver stained nucleolus-organizer regions of *P. pythagoricus*, *P. rapidus* (Keyserling 1880) and *P. punctulatus* Mello-Leitão 1944 are described and compared. In *P. pythagoricus* the identification of the chromosome pairs by means of G-banding is also performed. Females of the three species show a chromosome complement of 44 telocentric chromosomes, with a similar karyotype. Males of *P. pythagoricus* show 42 telocentric chromosomes, the two sex chromosomes being the largest and of different size. In the three species, two pairs of telomeric NORs and small pericentromeric positive C-bands in all chromosomes were detected. This C-banding pattern seems to be characteristic of spiders. Comparative analysis of chromosome complements in Sparassidae indicates that $2n = 42/44$ ($X_1X_20/X_1X_1X_2X_2$) (male/female) may represent the ancestral karyotype for *Polybetes*.

Keywords: Chromosome number, telocentric chromosomes, heterochromatin, nucleolus-organizing regions

Species of *Polybetes* are known exclusively from South America. To date there are thirteen described species, nine of them occurring in Argentina (Platnick 2006): *P. germaini* Simon 1897, *P. martius* (Nicolet 1849), *P. obnuptus* Simon 1896, *P. pallidus* Mello-Leitão 1941, *P. punctulatus* Mello-Leitão 1944, *P. pythagoricus* (Holmberg 1875), *P. quadrifoveatus* (Järvi 1914), *P. rapidus* (Keyserling 1880), and *P. trifoveatus* (Järvi 1914). In nature, they are found under the bark of trees (e.g., *P. pythagoricus* is common under the bark of *Eucaliptus*), in the branches of trees (*P. rapidus*), and others are found in grasses such as *Cortaderia* spp. (*P. punctulatus*). *Polybetes pythagoricus* and *P. rapidus* are also common in

cities where they are found in parks, gardens or even in the roofs of buildings. They are nocturnal and sometimes enter houses on stormy days. Despite their usual aggressiveness, they possess venom of low toxicity that causes little local injury and is harmless to humans (Scioscia, personal observations).

Cytogenetic studies in spiders are scarce, with only approximately 1% of nearly 39,500 described species determined. Within the Sparassidae, 36 species out of 1,009 (< 4%) had been previously cytogenetically analyzed; male diploid chromosome numbers range from 21 to 44; female diploid chromosome number is only known for four species: *Heteropoda venatoria* (Linnaeus 1767) ($2n = 44$); *Pediana regina* (L. Koch 1875), *Isopeda* sp. and *Olios* sp. ($2n = 46$). The most frequent sex chromosome determination system is $X_1X_2X_30/X_1X_1X_2X_2X_3X_3$ (male/female) (Hackman 1948; Suzuki 1950; Suzuki & Okada 1950; Bole-Gowda 1952; Suzuki 1952; Mittal 1961; Díaz & Sáez 1966a, b; Mittal 1966; Benavente & Wettstein 1978; Olivera 1978; Datta & Chatterjee 1983; Rowell 1985; Srivastava & Shukla 1986; Parida & Sharma 1986, 1987; Rowell 1991a, b; Hancock & Rowell 1995; Platnick 2006). Within the genus *Polybetes*, only *P. pythagoricus* had been previously cytogenetically analyzed (Díaz & Sáez 1966a, b; Benavente & Wettstein 1978; Olivera 1978).

There are only a few studies in spiders that characterize banding patterns of chromosomes; the distribution of C heterochromatin is known in eleven Sparassidae, eight Araneidae, five Lycosidae, four Tetragnathidae, two Nephilidae, two Sicariidae, one Scytodidae, and one Salticidae species, and G-banding was performed only in *Lycosa thorelli* (Keyserling 1877) (Lycosidae) and *P. pythagoricus* (Brum-Zorrilla & Cazenave 1974; Olivera 1978; Brum-Zorrilla & Postiglioni 1980; Rowell 1985; Datta & Chatterjee 1988; Rowell 1991b; Gorlova et al. 1997; Silva et al. 2002; De Araujo et al. 2005a, b).

In the present work, the karyotype, heterochromatin content and distribution, and silver stained nucleolus-organizer regions (NORs) of *Polybetes pythagoricus*, *P. rapidus* and *P. punctulatus* are described and compared. Furthermore, in *P. pythagoricus* the identification of the chromosome pairs by means of G-banding was performed.

METHODS

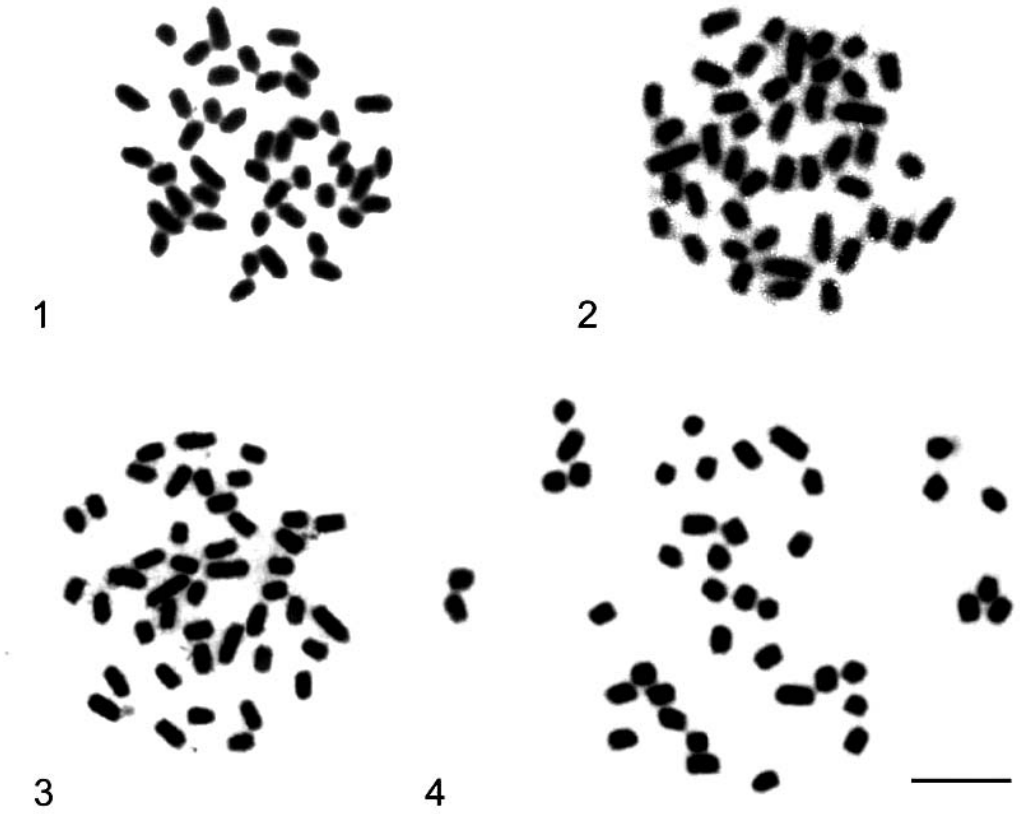
Adult females and males were collected in the field and were bred at the Arachnology Division of the Museo Argentino de Ciencias Naturales ''Bernardino Rivadavia'' (MACN). Voucher specimens of all species in this study have been deposited in the National Collection of Arachnology (MACN-Ar, Museo Argentino de Ciencias Naturales ''Bernardino Rivadavia'', Cristina Scioscia): *Polybetes pythagoricus*, five females and three males from Buenos Aires City, 34°35′15″S, 58°40′21″W, and Buenos Aires Province (Los Polvorines, 34°30′00S, 58°41′00″W; Villa Madero, 34°42′00″S, 58°30′00″W; San Isidro, 34°28′15″S, 58°31′43″W; and Martín García Island Natural Preserve, 34°11′15″S, 58°16′52″W); *P. rapidus*, six females from Buenos Aires Province (Bella Vista, 35°14′00″S, 59°53′00″W; Merlo, 34°40′12″S, 58°45′10″W; Villa Madero and Martín García Island Natural Preserve); *P. punctulatus*, one female from Martín García Island Natural Preserve and two immature females born in the lab.

For cytogenetic preparations, the specimens were cooled; and injected with 0.1 ml of 0.01% colchicine solution. After 1.25 h, several drops of hemolymph were removed from the coxal joints, and gonads together with some digestive tissues were dissected. Each sample was dispersed in 2 ml of hypotonic solution (KCl 0.56%) for 15 min, centrifuged at 800 rpm for 5 min, and fixed in 1 ml of 3 : 1 (methanol : acetic acid). The cell suspension was dropped onto clean slides, air-dried and stained with Giemsa 3% for chromosome counts and karyotyping.

C-band preparations were made following Sumner (1972) with some modifications: 0.2 N HCl for 1 h; saturated solution of $Ba(OH)_2$ for 30 s–1 min; 2 × SSC at 60° C for 1 h. Slides were air-dried and stained with 3% Giemsa.

G-bands preparations were made as follows: PBS solution for 15 min; 0.1% trypsin for 45 s–1 min. Slides were air-dried and stained with 3% Giemsa. NOR-banding was performed following Howell & Black (1980).

Eight to fifteen well-spread mitotic metaphases were measured to determine the karyotype of each species. Chromosome measurements were made using the computer



Figures 1–4.—Mitotic metaphases in spider chromosomes. 1. *Polybetes rapidus* female ($2n = 44$); 2. *P. punctulatus* female ($2n = 44$); 3. *P. pythagoricus* female ($2n = 44$); 4. *P. pythagoricus* male ($2n = 42$). Scale = 10 μm.

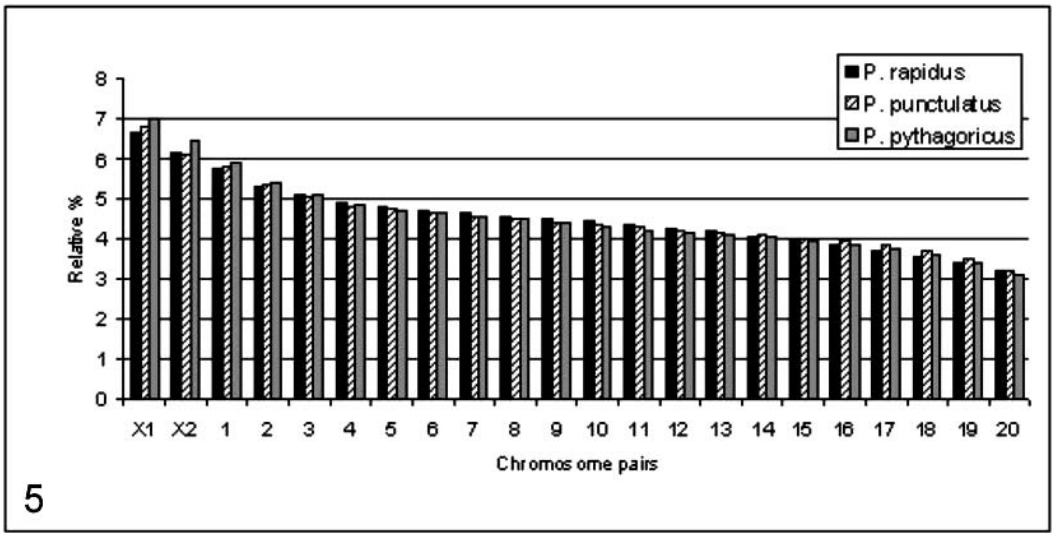
application Micromeasure version 3.3 (Reeves & Tear 2000). The total haploid complement length (TCL) in females was calculated by adding the mean value of each chromosome pair (in arbitrary units). In males of *P. pythagoricus*, the relative length of all chromosomes was analyzed to identify the two chromosomes that have no homologues (sex chromosomes), and TCL was afterwards calculated. The idiogram of each species was drawn on the basis of the relative percentage of each chromosome pair length to the TCL. Chromosome measurements were also made using a vernier calliper to estimate TCL in microns.

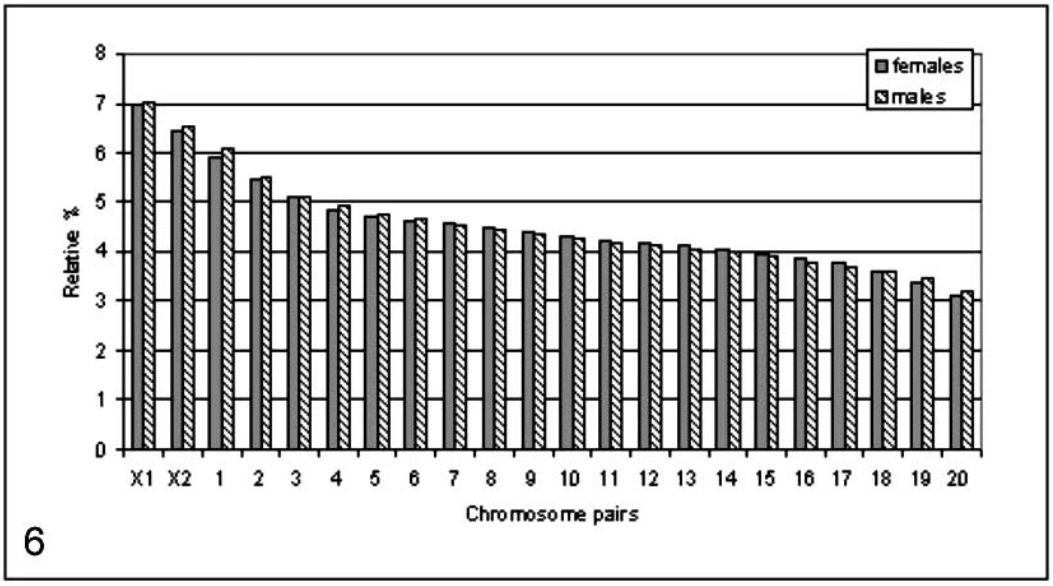
RESULTS

Chromosome complement.—Females of *P. rapidus*, *P. punctulatus* and *P. pythagoricus* show a chromosome complement of 44 telocentric chromosomes, and 42 telocentric chromosomes in males of *P. pythagoricus*. The sex chromosomes cannot be distinguished by their differential pycnosis in somatic metaphases of males and females (Figs. 1–4).

The total haploid complement length (TCL) is similar in the three species: 67.29 ± 4.91 μm in *P. rapidus*, 66.28 ± 6.91 μm in *P. pythagoricus* and 63.70 ± 1.52 μm in *P. punctulatus*.

Females of the three species show a similar karyotype: there are three large pairs of differently-sized chromosomes that can be distinguished; the rest of the chromosomal complement gradually decreases in size, except for the last pair that is slightly smaller. The largest chromosome pair shows slight size differences in the three species (*P. pythagoricus*, 7.00%; *P. punctulatus*, 6.80%; and *P. rapidus*, 6.63%), while the second pair is longer in *P. pythagoricus* (6.45%) (*P. punctulatus*, 6.12%; and *P. rapidus*, 6.16%) (Figs. 5, 7–9). In

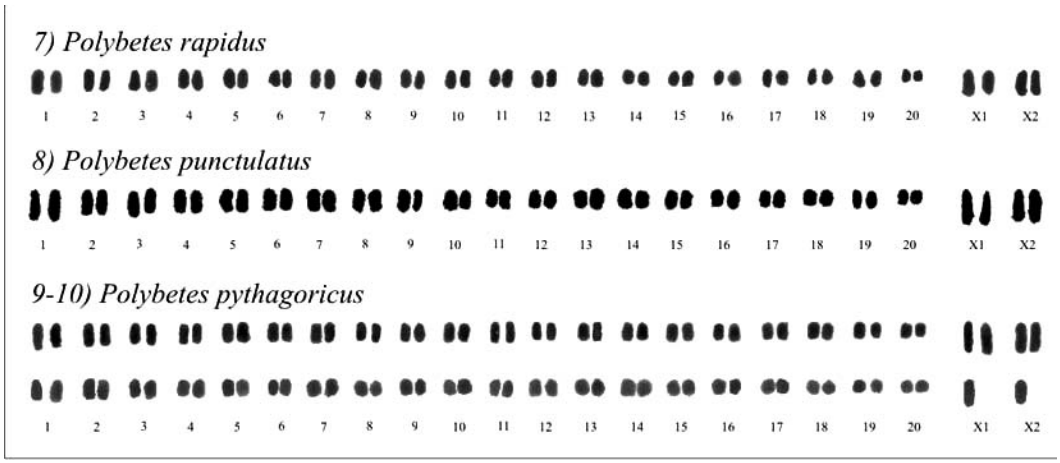




Figures 5, 6.—Comparative ideograms of spider chromosomes. 5. Females of *Polybetes rapidus*, *P. punctulatus* and *P. pythagoricus*; 6. Female and male of *P. pythagoricus*.

males of *P. pythagoricus*, the length analysis of all chromosomes of the complement makes it possible to determine that the two largest chromosomes of different sizes are the sex chromosomes X_1X_2 (Figs. 6, 10). Meiotic studies performed in males of *P. punctulatus* and *P. rapidus* (Rodríguez Gil 2006) demonstrated that in these species the sex chromosomes are also the largest of the complement.

C-banding and NORs silver staining.—In females of the three species, small positive C-bands in the pericentromeric region of all chromosomes were detected, except in the X_2 pair of *P. pythagoricus* where they are more prominent (Figs. 11, 12). The number of chromosomes with telomeric nucleolus-organizer regions (NORs) silver stained varied from 1 to 4 in different cells of the three species (Figs. 13, 14). It was not possible to determine precisely the NOR pairs; one pair was medium sized and the other was among the smaller ones.



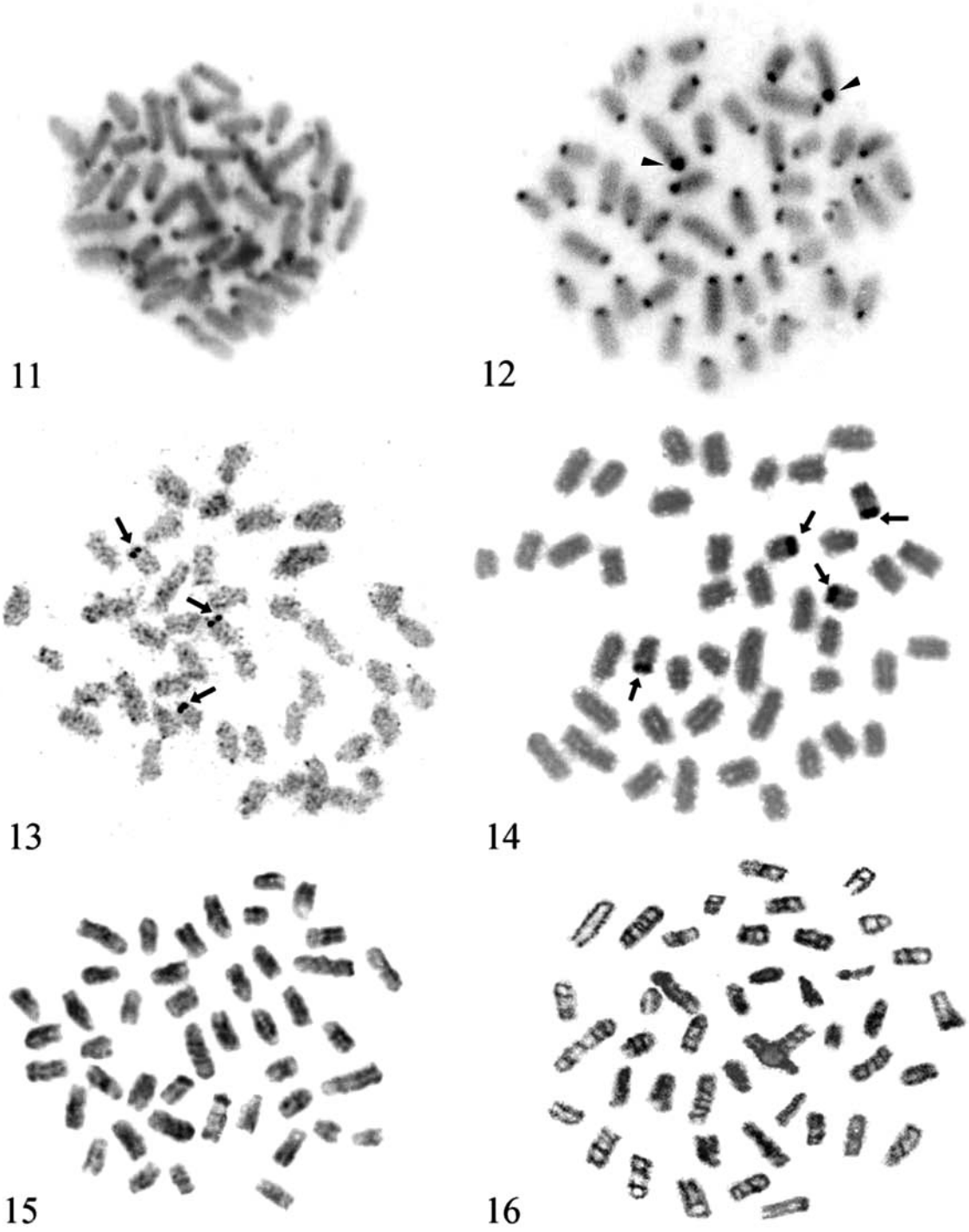
Figures 7–10.—Karyograms from spider cells depicted in Figures 1–4. 7. *Polybetes rapidus* female; 8. *P. punctulatus* female; 9. *P. pythagoricus* female; 10. *P. pythagoricus* male.

G-banding.—Despite applying the G-banding technique to the three species, only *P. pythagoricus* females yielded reproducible results, making it possible to determine the chromosome pair's identification (Figs. 16, 17). In *P. punctulatus*, only a few dark bands, with little contrast, were obtained in all the chromosomes (Fig. 15); therefore, the chromosome pair's identification was not possible. In *P. rapidus* no bands were obtained.

DISCUSSION

Chromosome complement and karyotype.—*Polybetes punctulatus* and *P. rapidus* were here cytogenetically characterized for the first time. *Polybetes pythagoricus* had been previously analyzed in Uruguayan populations; Benavente & Wettstein (1978) performed an ultrastructural study of sex chromosomes pairing in meiosis, and Díaz & Sáez (1966a, b) reported $2n = 42$ and $n = 20 + X_1X_2$ in males. However, Olivera (1978), in a preliminary report of *P. pythagoricus*, described contradictory data reporting a $2n = 40/42$ (male/female) with sex determination system $X_1X_2/X_1X_1X_2X_2$ at mitosis but in male meiosis described the presence of 10 chromosomes plus 2 Xs at one pole and 10 chromosomes at the other one in anaphase I. The three species of *Polybetes* analyzed in this work have $2n = 44 = 40 + X_1X_1X_2X_2$ in females and $2n = 42 = 40 + X_1X_2$ in *P. pythagoricus* males; they show very similar karyotype and total haploid complement length, with all the chromosomes telocentric. The sex chromosomes are the largest ones, the X_1 show slight size differences in the three species and X_2 is longer in *P. pythagoricus*. The conservative karyotype present in the three species could be considered characteristic for the genus.

Currently, cytogenetic studies in Sparassidae have been performed on 38 species from 17 genera (Table 1). Usually the chromosomes are telocentric and one of the sex chromosomes is the largest of the complement. The predominant diploid numbers in this family are $2n = 43$, $n = 20 + X_1X_2X_3$, 15 species; and $2n = 41$, $n = 19 + X_1X_2X_3$, 10 species. In other genera, besides *Polybetes*, there seems to be karyotypic conservation as in *Heteropoda* ($n = 19 + X_1X_2X_3$, in 5 of 6 species studied) and *Isopeda* ($n = 20 + X_1X_2X_3$, in the 4 species analyzed). Bole-Gowda (1952) stated that *Heteropoda sexpunctata* Simon 1885 has a derived karyotype, $2n = 20 + X$ (male) with 19 metacentric (including the X chromosome) and two acrocentric autosomes, on the basis of Robertsons law. On the other hand, in the genus *Sparassus*, there is variation not only in chromosome numbers ($2n = 22$ to 44) but in the sex chromosome determination system as well (X_1X_2, $X_1X_2X_3$, $X_1X_2X_3X_4$); although none of the entities was identified at the species level, and it is possible that the genus may be misidentified in some of them (Parida & Sharma 1987). An-



Figures 11–16.—C-banding in spider chromosomes: 11. *Polybetes punctulatus* female; 12. *P. pythagoricus* female (arrowheads point to prominent C-bands). NORs silver staining in spider chromosomes: 13. *P. rapidus* female; 14. *P. pythagoricus* female (arrows point to NOR regions). G-banding in spider chromosomes: 15. *P. punctulatus* female; 16. *P. pythagoricus* female. Scale = 10 μm.

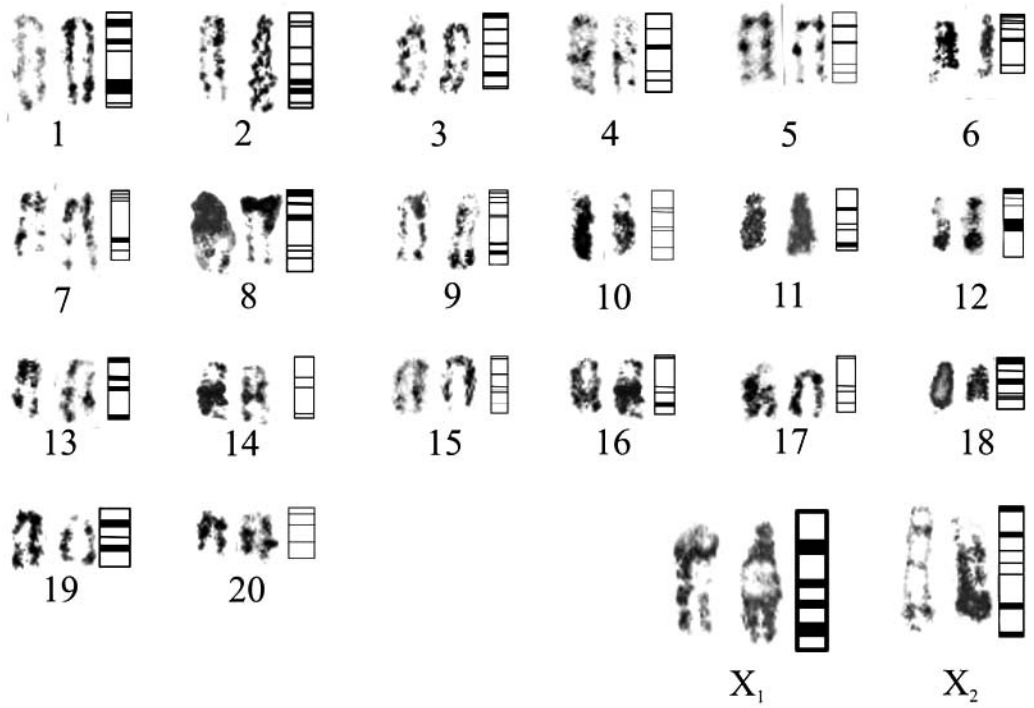


Figure 17.—G-banding karyogram and idiogram of *Polybetes pythagoricus* chromosomes (from cell depicted in Fig. 16).

cestral populations of *Delena cancerides* Walckenaer 1837 also show $n = 20 + X_1X_2X_3$, but this species has a number of chromosomal races that differ by the presence of particular combinations of chromosomal fusions, either in homozygous or heterozygous condition (Rowell 1985, 1990, 1991a, b; Hancock & Rowell 1995). A reduced complement is also present in *Micrommata virescens* (Clerck 1757), but neither the chromosome number nor the sex chromosome complement is known with certainty (Hackman 1948).

Heterochromatin characterization.—The three species of *Polybetes* analyzed here show only small pericentromeric heterochromatic bands in all the chromosomes with no differential pycnosis of the sex chromosomes, although Olivera (1978) reported that ''substantial'' heterochromatic blocks were present in *P. pythagoricus* mitotic and meiotic chromosomes. *Polybetes pythagoricus* X_2 chromosomes showed a larger C-band than in the other two species, which may explain the differences in these chromosomes' size.

Since the pioneer characterization of *Schizocosa malitiosa* (Tullgren 1905) (Lycosidae) by Brum-Zorrilla & Cazenave (1974), few spider species have been analyzed with regard to the heterochromatin content and distribution. Our results fit with previous data of most of the other spider species analyzed that have a small amount of pericentromeric heterochromatin in the autosomes and sex chromosomes; this condition seems to be characteristic in spiders. In *Loxosceles intermedia* Mello-Leitão 1934 (Sicariidae) and *Isopeda* species, pericentromeric C-bands are more conspicuous (Brum-Zorrilla & Postiglioni 1980; Rowell 1985, 1991b; Datta & Chatterjee 1988; Gorlova et al. 1997; Silva et al. 2002). In a few species, telomeric localization of heterochromatin (telomeric C-bands) has also been described in some chromosomes of the complement; these bands have usually appeared in a polymorphic condition (Rowell 1985; Datta & Chatterjee 1988; Rowell 1991b; De Araujo 2005a). In *Nephilengys cruentata* (Fabricius 1775) (Nephilidae) interstitial C-bands are present in some autosomes; the same occurs in some autosomes and the sex chromosomes of one unidentified species of *Scytodes* (De Araujo et al. 2005a, b).

Table 1.—Karyotype characteristics and collecting locality of the Sparassidae species cytogenetically analyzed (f = female).

Species	2 n	n (male)	Locality	References
Bhutaniella sikkimensis (Gravely 1931)	42	19 + $X_1X_2X_3X_4$	India	Datta & Chatterjee 1983
Delena cancerides Walckenaer 1837 (ancestral karyotype)	43	20 + $X_1X_2X_3$	Australia	Rowell 1985, 1991a, b; Hancock & Rowell 1995
Delena sp.	43	20 + $X_1X_2X_3$		McIntosh *in* Suzuki 1952
Heteropoda leprosa Simon 1884	41	19 + $X_1X_2X_3$	India	Datta & Chatterjee 1983
Heteropoda phasma Simon 1897	41	19 + $X_1X_2X_3$	India	Srivastava & Shukla 1986
Heteropoda procera (L. Koch 1867)	41	19 + $X_1X_2X_3$	Australia	Rowell 1985
Heteropoda sexpunctata Simon 1885	21	10 + X	India	Bole-Gowda 1952
Heteropoda venatoria (Linnaeus 1767)	41–44 f	19 + $X_1X_2X_3$	India, Japan	Suzuki & Okada 1950; Bole-Gowda 1952; Srivastava & Shukla 1986
Heteropoda sp. nov.	41	19 + $X_1X_2X_3$	Australia	Rowell 1985
Holconia immanis (L. Koch 1867)	43	20 + $X_1X_2X_3$	Australia	Rowell 1991a, b (sub *Isopoda*)
Isopeda vasta (L. Koch 1867)	43	20 + $X_1X_2X_3$	Australia	Rowell 1991b (sub *Isopoda vaster* (sic))
Isopeda villosa L. Koch 1875	43	20 + $X_1X_2X_3$	Australia	Rowell 1991a, b (sub *Isopoda*)
Isopeda sp.	43–46 f	20 + $X_1X_2X_3$	Australia	Rowell 1985 (sub *Isopoda*)
Isopeda sp. nov.	43	20 + $X_1X_2X_3$	Australia	Rowell 1991b (sub *Isopoda*)
Isopedella leai Hogg 1903	43	20 + $X_1X_2X_3$	Australia	Rowell 1991b (sub *Isopoda tepperi* Hogg)
Micrommata virescens (Clerck 1757)	±35	16 + $X_1X_2X_3$ (?)	Finland	Hackman 1948 [sub *Micrommata viridissima* (De Geer)]
Neosparassus diana (L. Koch 1875)	43	20 + $X_1X_2X_3$	Australia	Rowell 1991b (sub *Olios)*
Olios lamarcki (Latreille 1806)	42	20 + X_1X_2	India	Bole-Gowda 1952
Olios sp. 1	43	20 + $X_1X_2X_3$		McIntosh *in* Suzuki 1952
Olios sp. 2	43–46 f	20 + $X_1X_2X_3$	Australia	Rowell 1985
Parapalystes whiteae (Pocock 1902)	43	20 + $X_1X_2X_3$	India	Mittal 1961, 1966 (sub *Palystes*)
Pediana regina (L. Koch 1875)	43–46 f	20 + $X_1X_2X_3$	Australia	Rowell 1985, 1991b
Pediana sp. nov.	43	20 + $X_1X_2X_3$	Australia	Rowell 1991b
Polybetes punctulatus Mello-Leitão 1944	44 f	20 + X_1X_2	Argentina	this work; Rodríguez Gil 2006
Polybetes pythagoricus (Holmberg 1875)	42–44 f	20 + X_1X_2	Uruguay	Díaz & Sáez 1966a, b (sub *P. pitagorica* (sic))
			Argentina	this work; Rodríguez Gil 2006
	40–42 f		Uruguay	Olivera 1978 (sub *P. pithagorius* (sic))
Polybetes rapidus (Keyserling 1880)	44 f	20 + X_1X_2	Argentina	this work; Rodríguez Gil 2006

Table 1.—Continued.

Species	2 n	n (male)	Locality	References
Pseudopoda prompta (O. P.-Cambridge 1885)	41	19 + $X_1X_2X_3$	India	Srivastava & Shukla 1986 (sub *Heteropoda*)
Sinopoda forcipata (Karsch 1881)	41	19 + $X_1X_2X_3$	Japan	Suzuki 1952 (sub *Heteropoda*)
Sparassus sp. 1	44	21 + X_1X_2	India	Parida & Sharma 1987
Sparassus sp. 2	42	20 + X_1X_2	India	Parida & Sharma 1987
Sparassus sp. 3	41	19 + $X_1X_2X_3$	India	Parida & Sharma 1986, 1987
Sparassus sp. 4	41	19 + $X_1X_2X_3$	India	Parida & Sharma 1987
Sparassus sp. 5	22	10 + X_1X_2	India	Parida & Sharma 1987
Sparassus sp. 6	42	20 + X_1X_2	India	Datta & Chatterjee 1983 (sub *Parassus* sp. 1)
Sparassus sp. 7	44	20 + $X_1X_2X_3X_4$	India	Datta & Chatterjee 1983 (sub *Parassus* sp. 2)
Sparassus sp. 8	42	20 + X_1X_2	India	Datta & Chatterjee 1983
Spariolenus tigris Simon 1880	41	19 + $X_1X_2X_3$	India	Bole-Gowda 1952
Thelcticopis severa (L. Koch 1875)	43	Possibly $X_1X_2X_3$	Japan	Suzuki 1950, 1952 (sub *Thelticopis* (sic))

In spermatogonial mitosis, after C-banding, sex chromosomes have shown two different patterns. In three species of Lycosidae and in *Delena cancerides* the sex chromosomes were more darkly stained than the autosomes, while there was no difference in the sex chromosomes and autosomes in *Isopeda* and Araneidae species. In the three *Polybetes* species presented here and in four araneids, there is also no difference in the sex chromosomes and autosomes in female somatic and gonial cells. In one species, *Schizocosa malitiosa*, only one X chromosome was notable in that it exhibited complete heterochromatinization (Brum-Zorrilla & Cazenave 1974; Brum-Zorrilla & Postiglioni 1980; Rowell 1985; Datta & Chatterjee 1988; Rowell 1991b).

NORs silver staining and G-banding.—The variation in the number of chromosomes per cell bearing nucleolus-organizer regions observed in *Polybetes* species is common in the Ag-technique. It is characteristic of silver staining that not all the NORs are usually silver stained in species with multiple NORs, but only those transcriptionally active during the preceding interphase (Sumner 2003). It can be concluded that two chromosomal pairs with telomeric NORs are present in the three species here analyzed. Although the identification of the NOR pairs should be regarded as tentative, it seems possible that they correspond to the same pairs in the three species. Only a pair of NORs was detected at early somatic stages of *P. pythagoricus* Uruguayan specimens (Olivera 1978).

G-banding allows the precise identification of homologues and facilitates karyotypic comparisons between related species. Although good quality G-bands can be produced in reptiles, birds, mammals, in some fishes and amphibians, and in a few plants, this method does not yield consistent results in invertebrate chromosomes and only in a few species of insects have well-defined G-bands been obtained. The difficulty in obtaining good quality G-bands in invertebrates may reflect differences in mitotic chromosome substructure, e.g., tight compaction of the chromatin compared to vertebrates (Lorite et al. 1996; Appels et al. 1998; Baldanza et al. 1999; Sumner 2003). In spiders, G-banding had been performed in three species of Lycosidae (but only *Lycosa thorelli* showed consistent G-banding), and in *P. pythagoricus* from Uruguay (where only a few pairs could be identified) (Olivera 1978; Brum-Zorrilla & Postiglioni 1980). In the present work, the identification of all chromosome pairs was possible in *P. pythagoricus*. Taking into account that the pattern of pachytene chromomeres resembles that of G-bands on the same chromosome (Sumner 2003), it would be interesting to perform a

comparative analysis of the chromomere pattern of the three species in order to know if the scarcity and absence of G-bands in *P. punctulatus* and *P. rapidus* respectively is due to structural differences or to technical procedures.

The three species of *Polybetes* here analyzed are easily distinguished by morphological characters, but they are very conservative karyotypically. This fact could be useful in future for the delimitation of genera in a systematic revision of the family.

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