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# KARYOTYPES OF THREE SPIDER SPECIES (ARANEAE: PHOLCIDAE: *PHYSOCYCLUS)*

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*Abstract.*—Three species of the spider genus *Physocyclus* (*P. californicus, P. enaulus, P.* sp.) were karyotyped using an air-drying, Giemsa staining method. All chromosomes were metacentric, with males being 2n = 15 (N = 7 + XO) and females being 2n = 16. The karyotyping method resulted in slide preparations which were countable five years post-fixing.

Members of the spider family Pholcidae are known cytologically by only four species. In each case, only males have been examined, and XO and $X_1X_2O$ sex-determination mechanisms have been illustrated or reported (Sharma et al., 1959; Cokendolpher and Brown, 1985). Of these four species, reliable chromosome counts are available for only two species. Previous researchers have noted the difficulty in properly preparing pholcid chromosomes for accurate counting (Painter, 1914; Suzuki, 1954). It is the purpose of this publication to record the karyotypes of two additional species including the first female karyotypes of two species. Additionally, data is provided on the stability of chromosome preparations made by an air-drying method.

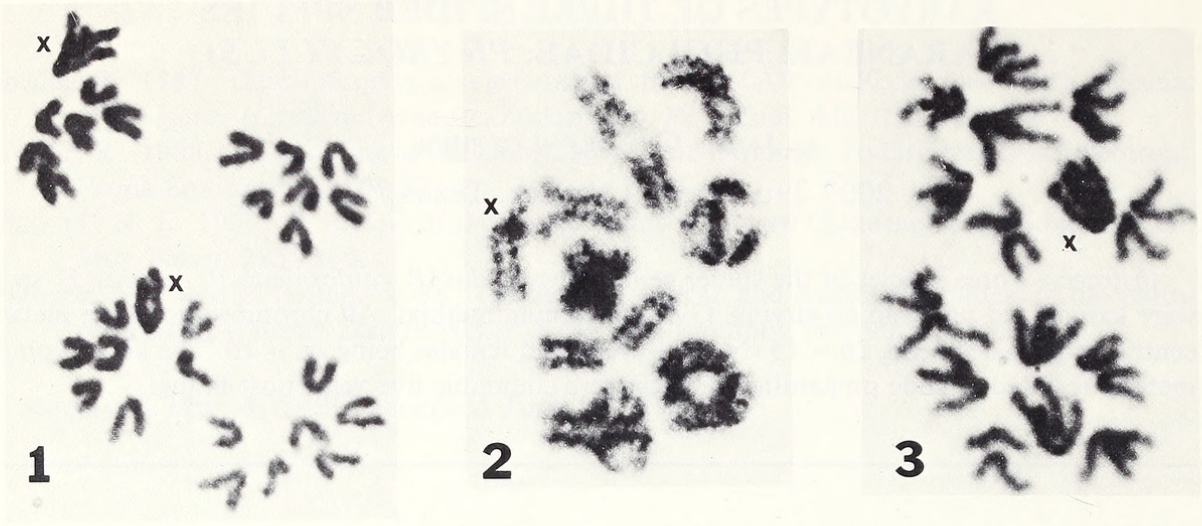
## MATERIALS AND METHODS

An adult male of *Physocyclus californicus* Chamberlin and Gertsch (9.5 km N Santa Isabella, San Diego Co., CA) and adult males and females of *P. enaulus* Crosby (Brackettville, Kinney Co., TX) and *P.* sp. (Lubbock, Lubbock Co., TX) were collected and returned to the laboratory. All specimens were dipped in a 0.005% colchicine/Ringer's solution and held for 24 hours to accumulate meiotic and mitotic metaphases. Two males of *P.* sp. were karyotyped without colchicine treatment to ascertain effects of the drug on chromosomes (see Smith, 1965; Sharma and Sharma, 1972). Testis and ovary preparations were air-dried and stained with Giemsa following the procedure outlined by Cokendolpher and Brown (1985). Preparations were not coverslipped and were maintained at room temperatures.

## RESULTS AND DISCUSSION

Chromosome preparations of a male *P. californicus,* two male *P. enaulus,* and four male *P.* sp. reveal 2n = 15, with n = 7 + XO (Figs. 1–4). These counts are based on 15, 14, and 31 nuclei, respectively, for the three species. Many meiotic nuclei were observed with 7:8 segregation with the X chromosome showing positive heteropycnosis (Fig. 1). During diakinesis the X's are isopycnotic (Fig. 2).

Chromosome preparations of two *P. enaulus* (nine nuclei) and three female *P.* sp. (15 nuclei) reveal 2n = 16. The sex chromosomes are indistinguishable from auto-




Figs. 1–3. Chromosomes of *Physocyclus* spp. males. 1. *P. enaulus* anaphase I. 2. *P.* sp. diakinesis. 3. *P.* sp. spermatogonial (?) metaphase.

somes during mitotic metaphase, except for the size of the X's. The three species of *Physocyclus* examined have only metacentric chromosomes.

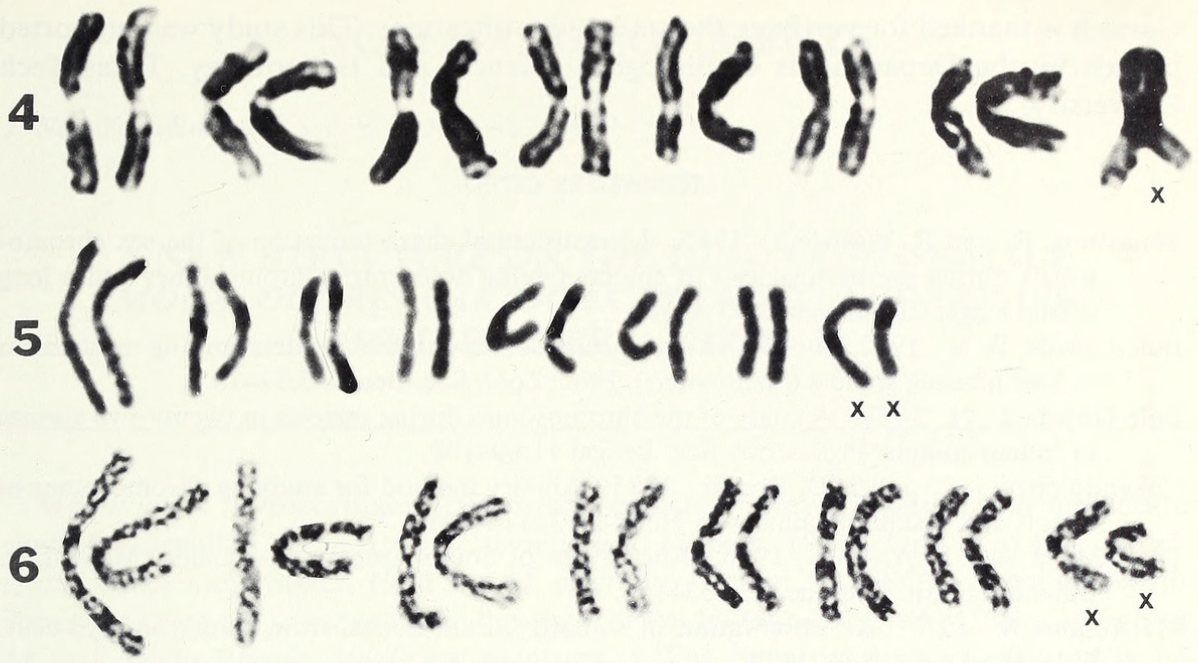
Bole-Gowda (1958) reported the haploid karyotype of *Crossopriza lyoni* (Blackwall) consisted of 13 metacentric autosomes and one metacentric X. In contrast, Sharma et al. (1959) recorded for the same species 11 metacentric autosomes and two acrocentric X chromosomes in the haploid set. Confirmation of this species' haploid elements is needed.

While Painter (1914) attempted to study *Pholcus phalangioides* (Fuessin) and *Spermophora meridionalis* Hentz, his admittedly unsatisfactory preparations revealed only the presence of two X chromosomes in the former species.

The haploid set of *Pholcus crypticolens* Bösenberg and Strand was reported by Suzuki (1954) to consist of 11 metacentric autosomes and two acrocentric X chromosomes.

Cokendolpher and Brown (1985:figs. 1–3) illustrated the chromosomes of male *P.* sp. but did not comment on females or the sex-determining mechanism. The chromosomes illustrated in that publication were from the same population as samples reported upon herein.

Male XO sex-determination mechanisms are uncommon in spiders (10% of 300 species) and are known only from ecribellate spiders: two species each from the Dysderidae and Segestriidae (Diaz and Saez, 1966; Benavente and Wettstein, 1980; Suzuki, 1954), two species of Lycosidae (Postiglioni and Brum-Zorrilla, 1981), eight species of Oxyopidae (Mittal, 1970), ten species of Thomisidae, one species each of Heteropodidae and Philodromidae (Bole-Gowda, 1952; Sokolow, 1962; Mittal, 1966), four species of Salticidae (Matsumoto, 1977), and possibly one Pholcidae (Sharma et al., 1959). Where the position of the centromere has been determined on spider species with XO males, all are acrocentric except for one Heteropodidae (Bole-Gowda, 1952; White, 1973) and possibly a Pholcidae (Sharma et al., 1959). In the case of *Heteropoda sexpunctata* (Simon) the male karyotype is 2n = 21 (19 metacentric and two acrocentric, with the X being metacentric). This rare condition is apparently




Figs. 4–6. Chromosomes of *Physocyclus* spp. 4. *P. californicus* male (2n = 15). 5. *P. enaulus* female (2n = 16). 6. *P.* sp. female (2n = 16).

normal for the genus *Physocyclus* since all three species examined showed a male XO sex-determination system.

While metacentric autosomes are rare in spiders (Sharma et al., 1959), the presence of metacentrics in both the autosomes and sex chromosomes has been recorded only in *Heteropoda sexpunctata* by Bole-Gowda (1952) and the questionable record by Bole-Gowda (1958) in *Crossopriza lyoni.*

The air-drying, Giemsa staining method proved to be an effective method for preparing countable spreads. Previous researchers have noted difficulty in fixing and counting pholcid chromosomes. These problems are apparently overcome with the present method. Furthermore, preparations maintained for five years at room temperatures in dark slide boxes remained suitable for analysis. The chromosomes illustrated in Figure 4 were photographed three years post-fixing. The stability of the preparations may have been aided by the relatively arid environment of Lubbock. After examination the slides were tilted, allowing any excess immersion oil to run off the slides, but were otherwise not cleaned.

Colchicine treated cells produced many more metaphase nuclei than untreated cells. Polyploids with countable chromosome numbers were present in low frequency (less than 15% of male nuclei counted). No other differences were noted between treated and non-treated chromosomes.

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## LITERATURE CITED

Benavente, R. and R. Wettstein. 1980. Ultrastructural characterization of the sex chromosomes during spermatogenesis of spiders having holocentric chromosomes and a long diffuse stage. Chromosoma 77:69–82.

Bole-Gowda, B. N. 1952. Studies on the chromosomes and the sex-determining mechanism in four hunting spiders (Sparassidae). Proc. Zool. Soc. Bengal 5:51–70.

Bole-Gowda, B. N. 1958. A study of the chromosomes during meiosis in twenty-two species of Indian spiders. Proc. Zool. Soc. Bengal 11:69–108.

Cokendolpher, J. C. and J. D. Brown. 1985. Air-dry method for studying chromosomes of insects and arachnids. Entomol. News 96(3):114–118.

Diaz, M. O. and F. A. Saez. 1966. Karyotypes of South-American Araneida. Mem. Inst. Butantan Simp. Internac. 33:153–154.

Matsumoto, S. 1977. An observation of somatic chromosomes from spider embryo-cells. Acta Arachnol. 27:167–172.

Mittal, O. P. 1966. Karyological studies on Indian spiders. IV. Chromosomes in relation to taxonomy in Eusparassidae, Selenopidae and Thomisidae. Genetica 37:205–234.

Mittal, O. P. 1970. Karyological studies on the Indian spiders. VIII. Chromosomes in male germ cells of three species of the genus *Oxyopes* (Family Oxyopidae). Microscope 1970: 313–318.

Painter, T. S. 1914. Spermatogenesis in spiders. J. Zool. Jahrb. Jena, Abt. Anat. 38:509–576.

Postiglioni, A. and N. Brum-Zorrilla. 1981. Karyological studies on Uruguayan spiders. II. Sex chromosomes in spiders of the genus *Lycosa* (Araneae-Lycosidae). Genetica 56: 47–53.

Sharma, G. P., B. L. Gupta and R. Parshad. 1959. Cytological studies on the Indian spiders. III. An analysis of the chromosomes in the male germ cells of the spider, *Crossopriza lyoni* (Blackwall), fam. Pholcidae. Res. Bull. Panjab Univ., N.S. Sci. 10:49–53, figs. 1–16.

Sharma, A. K. and A. Sharma. 1972. Chromosome Techniques Theory and Practice. Butterworth and Co., London, 2nd ed., 575 pp.

Smith, S. G. 1965. Heterochromatin, colchicine, and karyotype. Chromosoma 16:162–165.

[Sokolow, I. I.] 1962. [Studies on nuclear structures in Araneina. II. The sex chromosomes.] Tsitologia 4:617–625 (in Russian).

Suzuki, S. 1954. Cytological studies in spiders. III. Studies on the chromosomes of fifty-seven species of spiders belonging to seventeen families, with general considerations on chromosomal evolution. J. Sci. Hiroshima Univ., ser. B, div. 1, 15:23–136, pls. 1–15.

White, M. J. D. 1973. Animal Cytology and Evolution. University Press, Cambridge, 3rd ed., 923 pp.

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