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Cytogenetics of three species of scorpions of the genus *Brachistosternus* from Argentina (Scorpiones: Bothriuridae)

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Abstract. Meiotic studies on three phylogenetically distant species of the genus *Brachistosternus* Pocock from Argentina were conducted. One species is from the subgenus *Ministernus* Francke 1985, *B. ferrugineus* Thorell 1876, and two species are from the subgenus *Brachistosternus* Pocock 1893, *B. montanus* Roig-Alsina 1977 (Andean species group), and morphologically different populations of *B. pentheri* Mello-Leitão 1931 (plains species group). All species showed achiasmatic meiosis, absence of heteromorphic bivalents, and bibrachial and monobrachial chromosomes of different sizes. Males of *Brachistosternus ferrugineus*, *B. montanus,* and one population of *B. pentheri* have $2n = 46$. Males of the typical populations of *B. pentheri* have $2n = 42$. These results suggest that *B. pentheri* may comprise two species.

Keywords: Achiasmatic meiosis, Neotropics

The family Bothriuridae contains about 150 described species; it shows a Gondwanan distribution and has diversified mainly in southern South America. The systematics of this family has been resolved satisfactorily and the taxonomic status of most genera and species is well defined (Prendini 2003; Ochoa 2004a; Ojanguren-Affilastro & Ramírez 2009), making it particularly suitable for studying patterns of cytogenetic evolution. Chromosome number in Bothriuridae varies between 28 and 50 (Piza de Toledo 1947; Ferreira 1968; Giacomozzi 1977). Cytogenetic analyses have been performed on seven species, four species from the genus *Bothriurus* Peters 1861, *Timogenes elegans* (Mello-Leitão 1931) and two species of *Brachistosternus* Pocock 1893 (*B. pentheri* Mello-Leitão 1931 and *B. alienus* Lönnberg 1898) (Piza de Toledo 1947; Ferreira 1968; Giacomozzi 1977). The present study focuses on exploring cytological diversity among species and populations of *Brachistosternus* from Argentina.

Brachistosternus is the most diverse genus of the family, with about 40 known species (Ochoa 2002, 2004b; Ochoa & Acosta 2002; Ojanguren-Affilastro 2003a, b, 2005a, b; Ochoa & Ojanguren-Affilastro 2007; Ojanguren-Affilastro et al. 2007a, b; Ojanguren-Affilastro & Scioscia 2007). It inhabits arid areas in the western and southern parts of South America, from Ecuador (Cekalovic 1969) to southern Argentinean Patagonia (Ojanguren-Affilastro 2003b). The genus is divided into two subgenera (Ojanguren-Affilastro & Ramírez 2009), namely *Brachistosternus* Pocock 1893 and *Ministernus* Francke 1985. Both subgenera are present in Argentina. The subgenus *Brachistosternus* includes two large groups, one including lowland or plains species and the other mountain species from the Andes from altitudes between 2500 and 4500 m asl.

Here we report a cytogenetic study of three phylogenetically distant species of *Brachistosternus* in order to reveal possible chromosome variations in the genus. *Brachistosternus ferrugineus* (Thorell 1876) was selected as a representative of the subgenus *Ministernus*. This species is widely distributed in central and northern Argentina, eastern Bolivia, Paraguay and possibly in southwestern Brazil (Maury 1974). Two species were selected as representatives of the subgenus *Brachistosternus*, one belonging to the Andean group and the other to the plains group. *Brachistosternus montanus* Roig-Alsina 1977 (Andean group) is restricted to high-altitude areas of the Andean region (2700 to 3500 m asl) in central-western Argentina, in the provinces of Mendoza, San Juan and La Rioja (Ojanguren-Affilastro 2003a; Roig-Alsina 1977). *Brachistosternus pentheri* Mello-Leitão 1931 (plains group) is also found exclusively in Argentina, with a widespread distribution from Salta province to the southern part of Buenos Aires province. The morph of the northernmost *B. pentheri* found in the provinces of La Rioja, Catamarca and Salta (here designated as the northern morph) differs slightly from the type material by larger size and less pronounced pigmentation (Roig-Alsina & Maury 1984; Ojanguren-Affilastro 2005b). Both morphs of *B. pentheri* were analyzed cytogenetically to determine whether they also differ in karyotype.

METHODS

Specimens.—*Brachistosternus ferrugineus*: The specimens belong to two populations from an area near the center of the known distribution of the species: three males from the locality of Chepes, La Rioja province, Argentina (31°21′00″S; 66°35′60″W), and three males from the locality of San Marcos Sierra, Córdoba province, Argentina (30°46′60″S; 64°39′00″W). *Brachistosternus montanus*: Four males were obtained from the locality of Laguna Brava, La Rioja province (28°25′50″S; 69°00′31.3″W). This population has been previously mentioned under the name *B. affinis montanus* (Ojanguren-Affilastro 2003a) because it shows minor morphological differences from the typical morph of the species, which is present in San Juan and Mendoza provinces. However, we consider this population to be the same species after examining many additional specimens.

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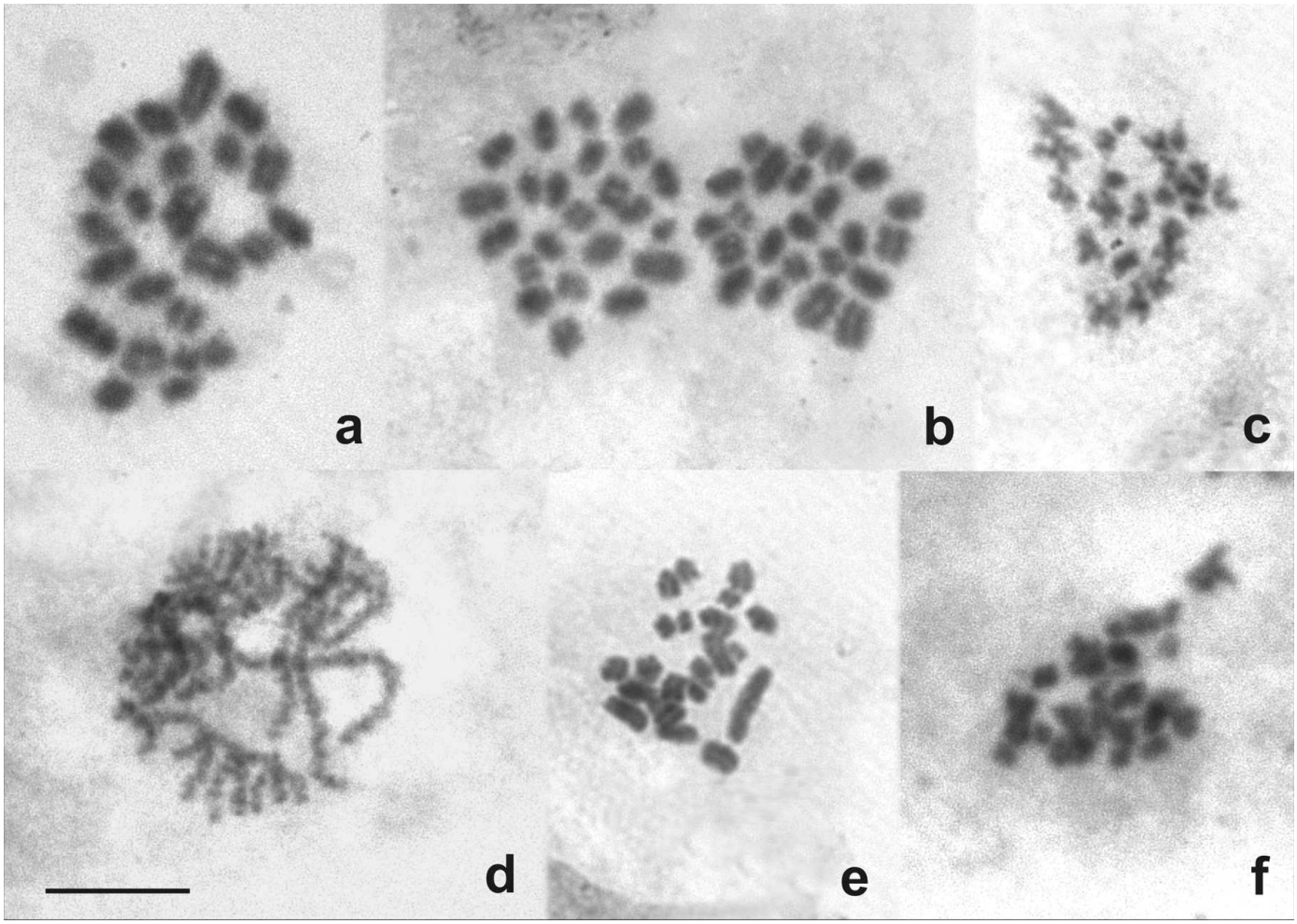

Figure 1. a–c.—*Brachistosternus ferrugineus* (n = 23). a. Late postpachytene; b. Two prometaphase I; c. Metaphase II.—d–f. *Brachistosternus montanus* ($2n$ = 46, n = 23). d. Pachytene; e. Prometaphase I; f. Prometaphase II. Scale bar: 10 μm.

Brachistosternus pentheri: Four males were obtained from the locality of Villa Unión, northern La Rioja province (29°18′00″S; 68°12′00″W) and belong to the northern morph. The typical morph was represented by three males from Chepes (southern La Rioja province) (31°21′00″S; 66°35′60″W), and one male from Oriente (coastal area of southeastern Buenos Aires province) (38°36′29″S; 60°37′07.3″W).

Cytogenetic methods.—All specimens were carried alive to laboratory and killed by cooling in a refrigerator. Their gonads were dissected in a physiological saline solution, swollen in hypotonic solution (0.56% KCl) for 10 min, and then fixed in a mixture of ethanol:chloroform:acetic acid (6:3:1). A piece of testis was placed on a slide, dissociated in a drop of 60% acetic acid with tungsten needles, and spread on the slide using a heating histological plate at approximately 45° C. Finally, the preparations were air-dried and stained with 3% Giemsa solution in water (pH = 7.4) for 10 min. Five postpachytene-prometaphase I of each cytotype of *B. pentheri* were measured to determine the meiotic karyotype. Bivalents measurements were made using the computer application Micromeasure version 3.3 (Reeves & Tear 2000). The relative length of each bivalent was calculated as a percentage of total haploid complement length (TCL). The idiogram of each cytotype was drawn on the basis of the relative percentage of each bivalent length to the TCL.

RESULTS

Subgenus *Ministernus* Francke 1985
(Figs. 1a–c; Table 1)

Brachistosternus ferrugineus has a karyotype of $2n$ = 46. No positively heteropycnotic bodies were observed at early prophase I. After pachytene, bivalents have no chiasma; homologous chromosomes lie parallel to each other, and condense gradually during prophase I and metaphase I. All bivalents are homomorphic (Figs. 1a, b). Chromosome plates of prometaphase and metaphase II consist of bibrachial (meta- or submetacentric) and monobrachial chromosomes of different sizes (Fig. 1c). No differences were observed between the specimens from the two localities.

Subgenus *Brachistosternus* Pocock 1893
(Figs. 1d–f, 2a–f, 3a–d; Table 1)

The chromosome complement of *B. montanus* consists of 46 chromosomes. At early prophase I no positively heteropyc-

Table 1.—Karyotype characteristics and collecting localities of the Bothriuridae species cytogenetically analyzed.

Species	2*n*	*n*	Locality	References
Bothriurus sp.	36	-	Três Lagoas, Matto Grosso, Brazil	Piza 1947
Bothriurus araguayae Vellard 1934	44	22	São Paulo, Brazil	Ferreira 1968 (sub. *B. asper araguaie*)
Bothriurus flavidus Kraepelin 1911	48	24	Buenos Aires Province, Argentina	Giacomozzi 1977
Bothriurus prospicuus Mello-Leitão 1934	50	25	Buenos Aires Province, Argentina	Giacomozzi 1977
Brachistosternus alienus Lönnberg 1898	28	14	Chubut Province, Argentina	Giacomozzi 1977
Brachistosternus ferrugineus (Thorell 1876)	46	23	San Marcos Sierra, Córdoba Province, Argentina	This work
B. ferrugineus	46	23	Chepes, La Rioja Province, Argentina	This work
Brachistosternus montanus Roig-Alsina 1977	46	23	Laguna Brava, La Rioja Province, Argentina	This work
Brachistosternus pentheri Mello-Leitão 1931	46	23	Villa Unión, La Rioja Province, Argentina	This work
B. pentheri	42	21	Chepes, La Rioja Province, Argentina	This work
B. pentheri	42	21	Oriente, Buenos Aires Province, Argentina	This work
B. pentheri	42	21	Buenos Aires Province, Argentina	Giacomozzi 1977(sub. *B. psammophilus* Maury)
Timogenes elegans (Mello-Leitão 1931)	48	24	Río Negro Province, Argentina	Giacomozzi 1977

notic bodies are present (Fig. 1d). Meiosis is achiasmatic; all bivalents are homomorphic, condensing gradually until metaphase I (Fig. 1e). Bibrachial and monobrachial chromosomes of different sizes were observed at prometaphase and metaphase II (Fig. 1f).

Morphs of *B. pentheri* showed different chromosome numbers. The karyotype of the northern morph (Villa Unión, northern La Rioja) exhibits 46 chromosomes (cytotype I) (Fig. 2), whereas karyotype of the typical morph from Chepes (southern La Rioja) and Oriente (southeastern Buenos Aires)

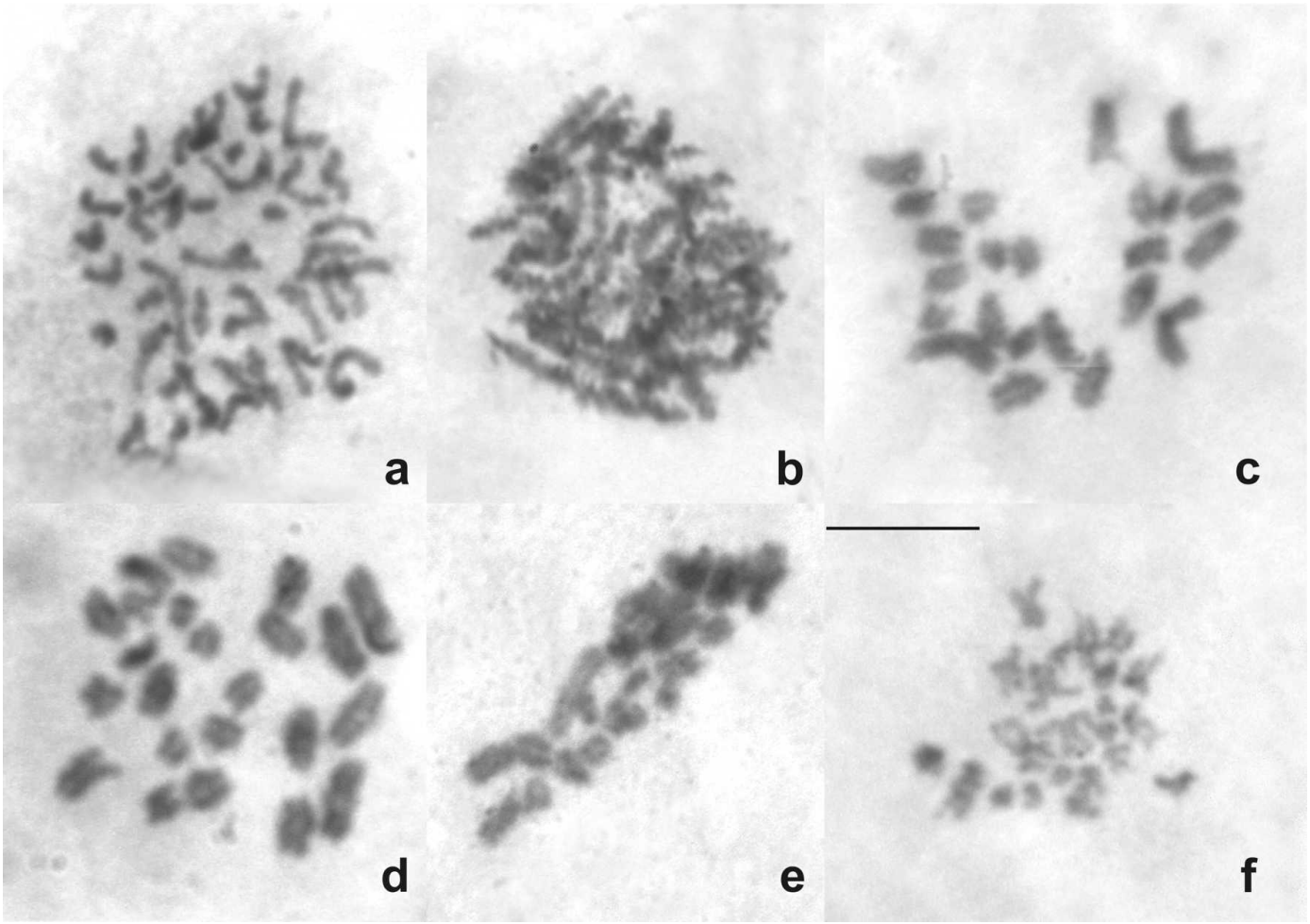

Figure 2. a–f.—*Brachistosternus pentheri* (2*n* = 46, *n* = 23). a. Spermatogonial prometaphase; b. Pachytene; c. Postpachytene; d. Prometaphase I; e. Metaphase I; f. Metaphase II. Scale bar: 10 μm.

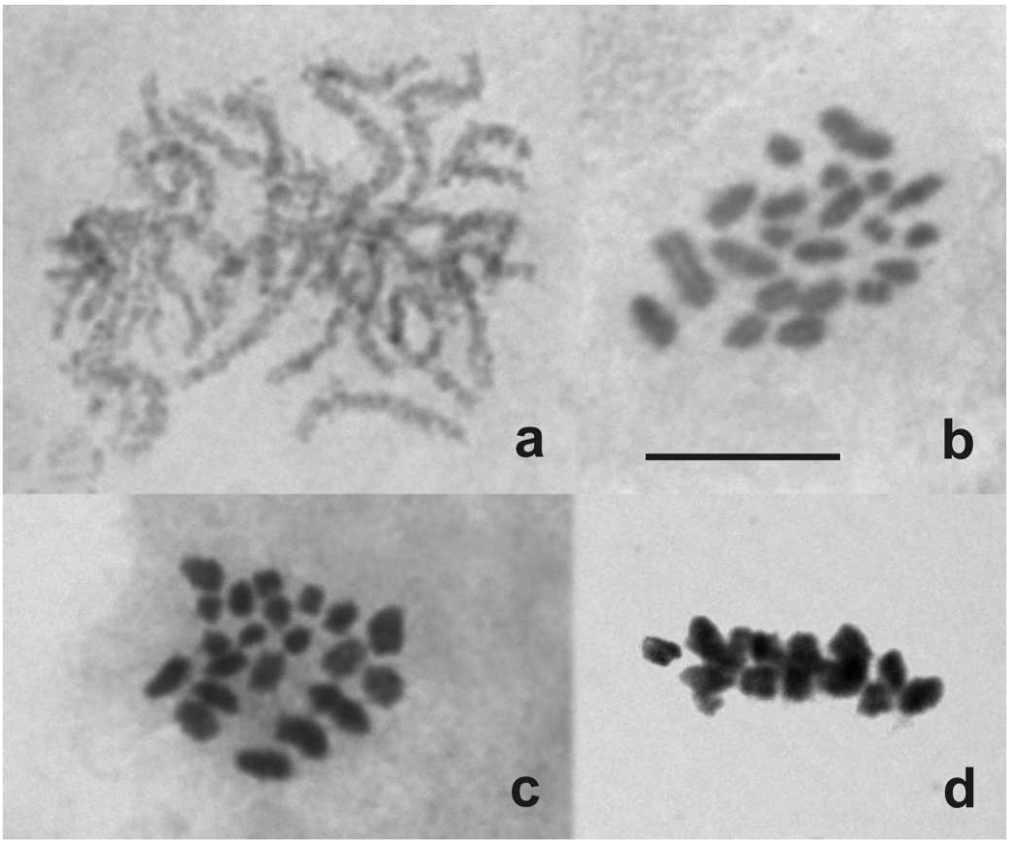

Figure 3. a–d.—*Brachistosternus pentheri* ($2n = 42$, $n = 21$). a. Pachytene; b. Late postpachytene; c. Prometaphase I; d. Metaphase I. Scale bar: 10 μm.

consisted of 42 chromosomes (cytotype II) (Fig. 3). Chromosomes of both morphs presented the same meiotic behavior. At spermatogonial prometaphases and metaphases chromosomes of different sizes were observed (Fig. 2a). At prophase I no positively heteropycnotic bodies were detected (Figs. 2b, 3a); meiosis is achiasmatic, and bivalents are homomorphic, condensing gradually during prophase I (Figs. 2c–e, 3b–d). Bibrachial (metacentric or submetacentric) and monobrachial chromosomes could be identified at metaphase II of individuals with $2n = 46$ (Fig. 2f). No metaphases II were observed in the individuals with $2n = 42$. Karyotype of *B. pentheri* cytotype I ($n = 23$) was formed by three larger bivalents of different size and the rest of the complement decreasing gradually in size (from medium-sized to small bivalents) (Fig. 4a, Table 2). Karyotype of *B. pentheri* cytotype II ($n = 21$) was formed by four larger bivalents of different size, 10 medium-sized bivalents that gradually decreased in size, and seven smaller bivalents that gradually decreased in size (Fig. 4b, Table 2).

DISCUSSION

This study focuses on cytogenetics of bothriurid scorpions of the genus *Brachistosternus*, comparing representatives of the subgenera *Ministernus* (*B. ferrugineus*) and *Brachistosternus* (*B. montanus* and *B. pentheri*). The karyotype of studied species is formed by a mixture of bibrachial and monobrachial chromosomes of different sizes. Meiotic complements of the three analyzed species of the family Bothriuridae contain no heteromorphic chromosome pair, which indicates the absence of heteromorphic sex chromosomes in males, as is also the case in *Buthus occitanus* (Amoreux 1789) and *Pandinus imperator* (C.L. Koch 1841) (Guénin 1957, 1961). Shanahan (1989a, 1989b) showed achiasmatic meiosis in males of Buthidae and Urodacidae. Our study of *Brachistosternus*, as well as that of Ferreira (1968) on *Bothriurus araguayae* Vellard 1934, reveals the presence of this derived type of meiosis also in Bothriuridae.

Our study provides the first cytogenetic analysis of *B. ferrugineus* and *B. montanus*. The karyotypes of these species consist of 46 chromosomes, a number that has not been found in the family Bothriuridae previously (Table 1). Although *B. ferrugineus* is widely distributed, there are almost no morphological differences between populations and no variation in chromosome number.

In contrast to *B. ferrugineus*, *B. pentheri* shows two different morphs (Roig Alsina & Maury 1984, Ojanguren-Affilastro 2005a), and our analysis revealed karyotypic differences between them. The northern morph of *B. pentheri* from Villa Unión (northern La Rioja) shows the same chromosome number as *B. ferrugineus*. In contrast, the karyotype of *B. pentheri* from Chepes (southern La Rioja) and Oriente (southeastern Buenos Aires) consists in 42 chromosomes. These specimens correspond morphologically to the species' holotype from Mendoza province, Argentina, and they belong to the typical morph of the species. Although the range of the

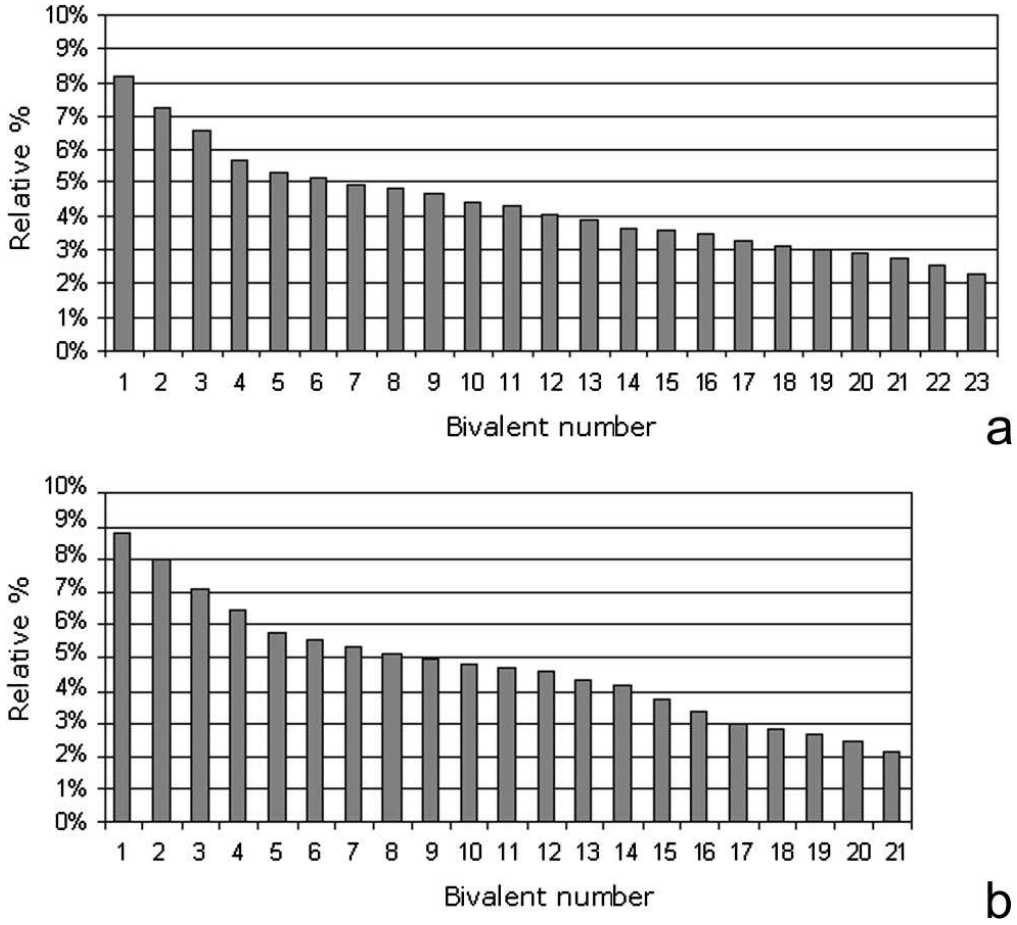

Figure 4. a–b.—Idiograms of *Brachistosternus pentheri* cytotypes. a. Cytotype I, $n = 23$; b. Cytotype II, $n = 21$.

relative lengths of the bivalents in both cytotypes was similar, three bivalent groups can be distinguished in cytotype II, but only two in cytotype I (Fig. 4). Since only 1% of *Brachistosternus* species were cytogenetically analyzed it was not possible to determine the ancestral karyotype and therefore the rearrangements that lead to the different chromosome numbers (e.g., centric or tandem fusions versus fissions). The intraspecific morphological and karyotypic variations of *B. pentheri* indicate that its marginal northern populations could be a different subspecies or even species. This proposal could be tested by attempting hybridization between the typical and northern morphs.

The genus *Brachistosternus* was studied cytogenetically for the first time by Giacomozzi (1977) (Tab. 1), who mentions that his specimens were collected and determined by Dr. E. Maury as *B. psammophilus* Maury 1977 and *B. alienus* Lönnberg 1898. *B. psammophilus* was considered a possible endemic species confined to coastal dunes in southern Buenos Aires province (Maury 1977). However, some years later Roig Alsina & Maury (1984) synonymized *B. psammophilus* with *B. pentheri*, a widespread species from central and northern Argentina. Therefore, the specimens studied by Giacomozzi (1977) as *B. psammophilus* should be considered *B. pentheri*. The karyotype of the specimens studied by Giacomozzi as *B. psammophilus* consisted of 42 chromosomes, like our specimens of *B. pentheri* from Oriente. Both samples of specimens belong to the same group of populations from coastal dunes of southern Buenos Aires.

On the other hand, the identity of the second species of *Brachistosternus* studied by Giacomozzi (1977) is uncertain. Maury determined these specimens as *B. alienus* (Giacomozzi 1977), but it is possible that they belong to *B. angustimanus* Ojanguren-Affilastro & Roig-Alsina 2001. At the time of Giacomozzi's investigation, most authors based the identification of *B. alienus* on the redescription by Mello-Leitão (1938, 1945), whose definition of *B. alienus* encompassed another species now known as *B. angustimanus* (Ojanguren-Affilastro 2001; Ojanguren-Affilastro & Roig-Alsina 2001). Both species are sympatric over most of their ranges, but *B. angustimanus* is more commonly found than *B. alienus* because of its larger size and higher abundance.

Brachistosternus alienus (*sensu* Giacomozzi 1977) shows the lowest chromosome number known for the genus ($n = 14$) (Giacomozzi 1977). Recent phylogenetic analyses (Ojanguren-Affilastro 2008; Ojanguren-Affilastro & Ramírez 2009) that include both morphological and molecular data placed *B. pentheri* as the sister group of the clade (*B. angustimanus* (*B. alienus* (*Brachistosternus telteca* Ojanguren-Affilastro 2000, *Brachistosternus multidentatus* Maury 1984))). Therefore, the assessment of whether the low chromosome number is a

Table 2.—Relative lengths (RL) of bivalents of *Brachistosternus pentheri* cytotypes.

Bivalent number	RL (%)	
	Cytotype I (n=23)	Cytotype II (n=21)
1	8.17	8.78
2	7.22	7.99
3	6.57	7.14
4	5.70	6.42
5	5.34	5.79
6	5.13	5.57
7	5.00	5.39
8	4.85	5.12
9	4.65	4.96
10	4.41	4.83
11	4.29	4.71
12	4.08	4.58
13	3.91	4.33
14	3.68	4.17
15	3.63	3.74
16	3.48	3.36
17	3.28	3.02
18	3.11	2.81
19	3.00	2.66
20	2.89	2.46
21	2.78	2.17
22	2.53	
23	2.30	

synapomorphy of the clade or an autapomorphy of the specimens determined by Maury as *B. alienus* should be made using specimens accurately identified as *B. alienus* and *B. angustimanus*.

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