Rev. Brasil. Genet. 13, 1, 19-31 (1990)
(Brazil. J. Genetics)

ON A NEW CAVE-DWELLING SPECIES OF BAT-GUANO-BREEDING *Drosophila* CLOSELY RELATED TO *D. repleta* WOLLASTON (DIPTERA, DROSOPHILIDAE).

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ABSTRACT

Drosophila (*Drosophila*) *eleonorae*, sp. n. (Type-locality: "Caverna Pedra da Cachoeira", Altamira, Pará, Brazil), a cave-dwelling fly is described, illustrated and compared to a closely related species, the cosmopolitan *D. repleta* Wollaston. Although morphologically distinct they are homosequential regarding their polytene chromosome banding pattern and have an identical constitutive heterochromatin distribution in the mitotic metaphase chromosomes. Adult specimens of *D. eleonorae*, sp. n. were collected in several caves located in five states of northern, central and southeastern Brazil (Pará, Goiás, Mato Grosso do Sul, Minas Gerais and São Paulo). Twenty-one imagines emerged in the laboratory from bat-guano collected in a cave of central Brazil. Attempts to produce interspecific hybrids with *D. repleta* were unsuccessful.

INTRODUCTION

The neotropical *Drosophila repleta* group consists of 80 described species (Vilela, 1983; Wassserman *et al.*, 1983; Fontdevila *et al.*, 1988; Rafael and Arcos, 1988 and Suyo *et al.*, 1988). Most of them are found in dry environments of Mexico, Central and South America, where they utilize species of Cactaceae as breeding sites. So far, all but 7 described species have been ascribed to five subgroups. In contrast with the largest (38 species) and desert-dwelling *D. mulleri* species subgroup, the members belonging to the *fasciola* subgroup (18 species) mostly inhabit the wet rain forests of

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South America. Up to now, the species ascribed to the remaining subgroups namely, *hydei* (seven species), *mercatorum* (four species) and *repleta* (six species) were not, as a whole, clearly associated to any typical environment.

According to Vilela (1983) the *repleta* subgroup comprises the following six species: *D. fulvimacula, D. fulvimaculoides, D. limensis, D. neorepleta, D. repleta* and *D. zottii*. We believe that *D. vicentinae*, a *repleta* group species previously not assigned to any subgroup, may prove to belong to this subgroup. It should be pointed out that Wasserman (1982) considered *D. peninsularis* chromosomally closer related to the *repleta* subgroup species than to those belonging in the *mercatorum* subgroup where we still believe that, morphologically, it seems to fit better.

The nominate species of this group, *D. repleta,* was described by Wollaston in 1858 from flies collected in the houses of Funchal, Madeira Island. Patterson (1943) pointed out that in the southwestern United States and northern Mexico, it is usually taken around fruit stores, toilets and urinals. In Brazil, we have also noticed that the imagines can be seen frequently in great numbers on the ceiling and walls of public toilets, especially the dirty ones. Currently, it is well known that *D. repleta* is a cosmopolitan species often associated with human activities. It is believed that, prior to becoming widespread all over the world, *D. repleta* originated from a primitive form somewhere in the New World where its five or so closest related species (belonging to the same subgroup) are endemic too.

Wasserman (1960, 1962a-d, 1982) has extensively studied and well documented the chromosomal evolution in the five recognized subgroups belonging to the *repleta* group. According to him, the *repleta* subgroup comprising 8 species, differs from the most probable ancestral sequence of the *repleta* group by five inversions ($X\bar{a}$, $X\bar{b}$, $X\bar{c}$, $2\bar{a}$ and $2\bar{b}$). He also recognized 3 species complexes (*fulvimacula, repleta* and *peninsularis*) in the *repleta* subgroup. One additional inversion (3b) is shared by all the species he included in the *repleta* complex except *D. limensis*, i.e., *D. repleta, D. neorepleta, D. melanopalpa* and *D. canapalpa*. Vilela (*in* Wheeler, 1981) considers the latter two binomials as synonymies of *D. repleta* and *D. neorepleta* respectively.

The discovery of a new species which will probably help to clarify the origin of its close relative, the cosmopolitan *D. repleta* with which it is chromosomally homosequential prompted the present paper.

MATERIAL AND METHODS

The flies used in the descriptiopn of the new species and to perform the hybridization tests were obtained from two isofemale strains. The wild-caught female which originated the strain F15F3 (*D. eleonorae*, sp. n.) was collected in a cave named "Caverna Pedra da Cachoeira", 20 Km SW of Altamira, state of Pará, Brazil and the female which gave origin to the strain E79F5 (*D. repleta*) was collected in a kitchen, downtown São Paulo City, state of São Paulo, Brazil. Both strains are currently being

maintained in the "Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo", São Paulo, Brazil.

Fifty-one specimens sampled by several collectors from different brazilian caves were also analyzed and are listed under the item "other specimens examined".

Third instar larvae were dissected for their ganglia and imaginal discs which were prepared according to a technique modified after Baimai (1977).

The dissection of the salivary glands and the method of chromosome squash preparation used were identical to those described in Yoon *et al.* (1973).

In preparing the male and female genitalia we followed Wheeler & Kambysellis (1966) and Kaneshiro (1969). The male genitalia terminology previously discussed by Vilela and Pereira (1985) is followed.

Drosophila repleta, Godoy, 1986:85, 89; Trajano, 1987: 537, 547, 551 (misidentification *non Drosophila repleta* Wollaston, 1858: 117).

Drosophila cf. *repleta*, Trajano, 1987: 547.

Drosophila aff. *repleta*, Gnaspini-Netto, 1989:

Drosophila sp., Trajano and Moreira (in press).

Type-Material. Holotype male, labelled "Brasil PA, 20 Km SW Altamira, Caverna Pedra da Cachoeira, 3°18'S, 52°20'W, E. Trajano col. 21-22.X.1988/DBIBUSP, estoque F15F3; VI. 1989/HOLÓTIPO *Drosophila eleonorae* ♂". Thirty-one paratypes (15 ♂, 16 ♀) same data as holotype. All type-specimens (except the original wild-caught female) were obtained in June 1989 from one isofemale line strain (F15F3). Genitalia of the holotype and four paratypes (1 ♂, 3 ♀) have been removed and dissected. The type-specimens are housed in the "Museu de Zoologia, Universidade de São Paulo" (MZUSP), São Paulo, Brazil. Type-locality "Caverna Pedra da Cachoeira", 20 Km SW Altamira (3°18'S, 52°20'W), Pará, Brazil.

Body length (pinned specimens) about 2.9mm (♂) and 3.4mm (♀).

Head. Arista with 4-5 dorsal and 2 ventral branches plus terminal fork. Antennae proximally light brown, distally brown. Front light brown, pollinose; posterior region darker, middle orbital about half of other two. Face light brown, pollinose. Carina slightly rounded, not sulcate, pollinose. Palpi light brown, pollinose, with bristles on ventral surface. Cheeks yellow, pollinose, their greatest width 1/3 greatest diameter of eyes. Eyes vermilion-like (wine colored in *D. repleta*) with short black piles.

Thorax. Acrostichal hairs in 8 irregular rows. Prescutellars absent. Anterior scutellars convergent. Mesonotum yellowish brown, pollinose, with most bristles arising from light brown spots, somewhat irregularly fused. Scutellum and pleurae light brown, pollinose. Sterno index about 0.7 (about 0.9 in *D. repleta*). Halteres light brown. Coxae, femora, tibiae and tarsi yellowish brown. Apical bristles on 1st and 2nd tibiae, preapicalls on all three. Wings slightly brownish. Costal index about 3.7 (about

3.0 in *D. repleta*); 4th vein index about 1.5; 5x index about 1.3; 4c index about 0.7; M index about 0.4. Apex of first costal section black, with two enlarged bristles. Third costal section with heavy bristles on its basal 1/2. Wing length about 2.7 mm (♂), 2.8 mm (♀).

Abdomen. Yellow, each segment with a large faintly interrupted light brown band (narrower, darker, widely interrupted and more distinct in *D. repleta*), which fades away laterally (in *D. repleta* it bends toward the anterior margin at angle of tergites, leaving a yellow lateral area).

Male Genitalia. Testes pale yellow with about 4 inner and 5 loose outer coils (4 inner and 11 tight outer coils in *D. repleta*). Epandrium (Figure 1A) with about 16 lower (about 13 in *D. repleta*) and 6 upper (about 3 in *D. repleta*) bristles. Cerci (Figure 1A) anteriorly fused at lower half. Surstylus (Figures 1A, 1B) not micropubescent with about 7 primary teeth (about 10 in *D. repleta*) and 7 marginal bristles. Hypandrium (Figure 1C) about 2/3 length of epandrium; gonopod bare with a wrinkly surface (smooth in *D. repleta*). Aedeagus (Figures 1D-1H, 2B, 2C) distally spatulate (pointed in *D. repleta*, Figures 2E, 2F) in profile, slightly invaginated at distal dorsal tip; dorsal region proximally rounded (pointed in *D. repleta*) and expanded upwards; dorsal cleft about 1/3 (about 1/6 in *D. repleta*) of length. Aedeagal apodeme rod-shaped, ventral rod slightly longer than paramere. Paramere with one sensillum. Phallosomal index about 1.1 (about 1.3 in *D. repleta*).

Female Genitalia. Ventral receptacle an irregular spiral with about 50 coils (about 90 in *D. repleta*). Ovipositor plate (Figure 1I) apically pointed with about 15 marginal and 5 discal teeth (respectively about 19 and 4 in *D. repleta*), ventral margin almost straight (convex in *D. repleta*, Figure 1J) Spermatheca (Figure 2A) olive-shaped, sclerotized with irregular circular ridges on proximal region and about 2/3 length of the finger-stall-shaped spermatheca of *D. repleta* (Figure 2D); duct deeply invaginated.

Eggs. Length about 0.6 mm (0.5 mm in *D. repleta*); 4 filaments about 1.2x egg length (about the same length as the egg in *D. repleta*).

Puparia. Reddish brown; horn index about 2.8 (about 2.6 in *D. repleta*), each anterior spiracle with about 17 branches.

Metaphase Chromosomes. *D. eleonorae*, sp. n. and *D. repleta* Wollaston were shown to have identical constitutive heterochromatin distribution in mitotic metaphase chromosomes. They have the basic number of six chromosome elements (2n = 12), consisting of 4 pairs of telocentric (rod-shaped) autosomes, 1 pair of small-dot autosomes (microchromosomes) and 1 pair of sex chromosomes. The rod-shaped autosomes are similar in size and ca. 2/3 length of X chromosome. The X chromosome is telocentric with ca. 1/4 of its proximal region composed of heterochromatin. The short telocentric Y chromosome is entirely hererochromatic and about as long as the heterochromatic region of X chromosome (Figures 3A-3D, 4A, 4B).

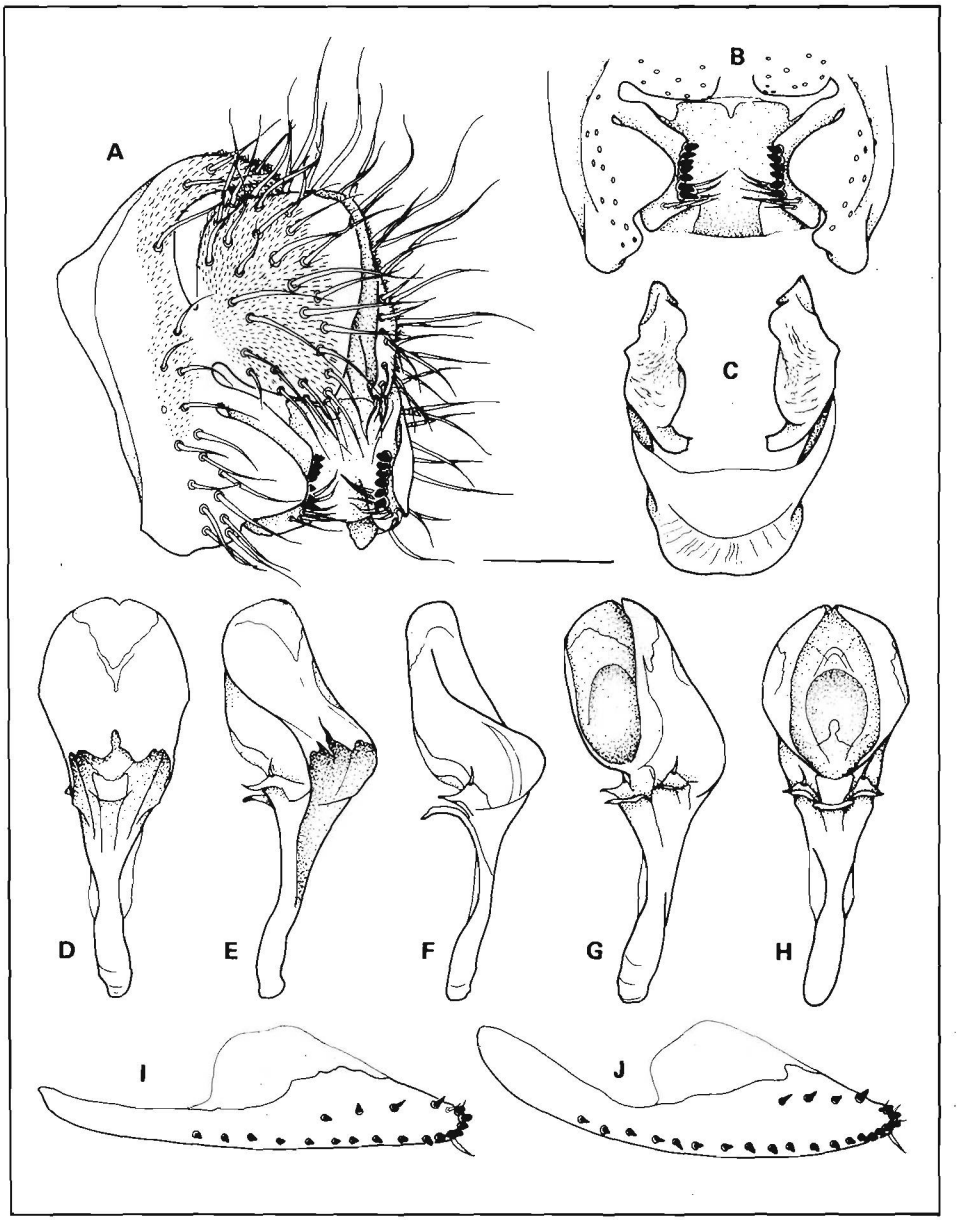

Figure 1 - (A-I) *Drosophila eleonorae*, sp. n. (♂, holotype; ♀, paratype): (A) epandrium, cerci and surstyli, latero-oblique aspect; (B) surstyli, posterior aspects; (C) hypandrium and gonopods, posterior aspect; (D-H) several views of aedeagus, parameres and aedeagal apodeme; (I) left ovipositor plate, lateral aspect. (J) *D. repleta* Wollaston (Strain E79F5, São Paulo, SP): left ovipositor plate, lateral aspect. Bar = 0.1 mm.

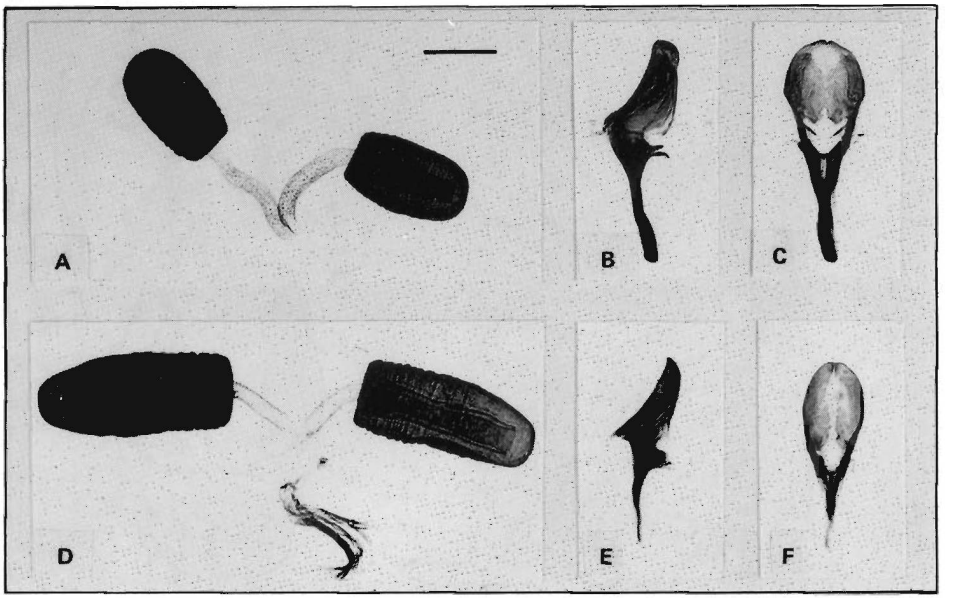

Figure 2 - (A-C) *Drosophila eleonorae*, sp. n. (♂, holotype, ♀ paratype): (A) spermathecae; (B) aedeagus, parameres and aedeagal apodeme, lateral aspect; (C) idem, dorsal aspect. (D-F) *Drosophila repleta* Wollaston (strain E79F5, São Paulo, SP): (D) spermathecae; (E) aedeagus, parameres and aedeagal apodeme, lateral aspect; (F) idem, dorsal aspect. Bar = 0.1 mm.

Polytene Chromosomes. *D. eleonorae*, sp. n. and *D. repleta* Wollaston have linearly identical polytene chromosome banding patterns, i.e., they represent a cluster of two homosequential species. This suggests that the changes involved in speciation are not evident in the polytene band sequence.

To facilitate comparisons the photographic maps of the salivary chromosomes of both *D. eleonorae* and *D. repleta* are also included (Figures 5, 6). The chromosome 6 (microchromosome) was intentionally omitted. The system of band designation employed in the map of *D. repleta* presented by Wharton (1942) has been used here.

Life Cycle (at room temperature). Larval stage about 11 days (about 9 in *D. repleta*), and pupal stage about 6 days (about 5 in *D. repleta*).

Hybridization Tests. Two groups (with 4 replicates) of crosses (*D. repleta* ♂ x *D. eleonorae* ♀ and *D. repleta* ♀ x *D. eleonorae* ♂) plus 2 replicates of the controls (*D. repleta* ♂ x *D. repleta* ♀ and *D. eleonorae* ♂ x *D. eleonorae* ♀) were performed in mass culture vials (about 7 virgin pairs to the vial), kept at room temperature. The pairs were transferred daily to fresh food vials during a period of 30 days. Although one interspecific mating (*D. repleta* ♂ x *D. eleonorae* ♀) has been observed, the crosses yielded no larva.

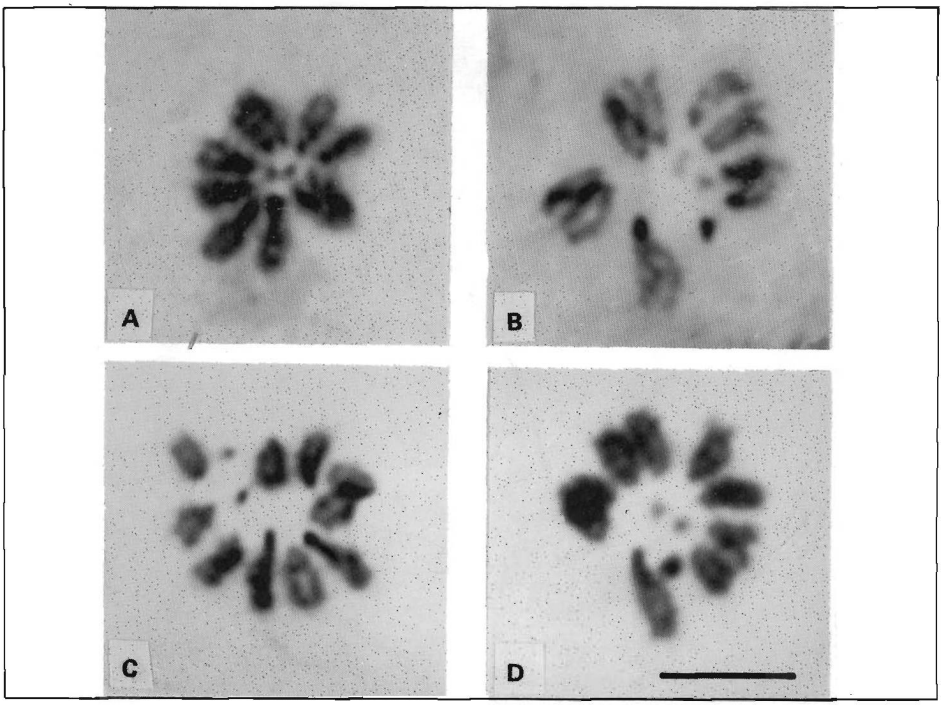

Figure 3 - (A-D) Photomicrographs male and female metaphase plates of *Drosophila repleta* Wollaston (A, ♀; B, ♂) and *Drosophila eleonorae*, sp. n. (C, ♀; D, ♂). Heterochromatic parts of each chromosome are darkly stained. Bar = 5 μm.

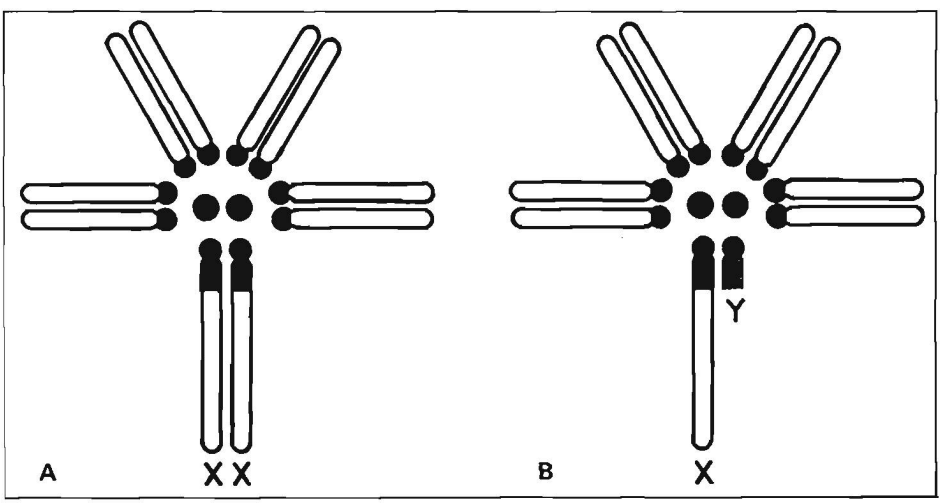

Figure 4 - Karyotypes of female (A) and male (B) of *Drosophila eleonorae*, sp. n. and *Drosophila repleta* Wollaston.

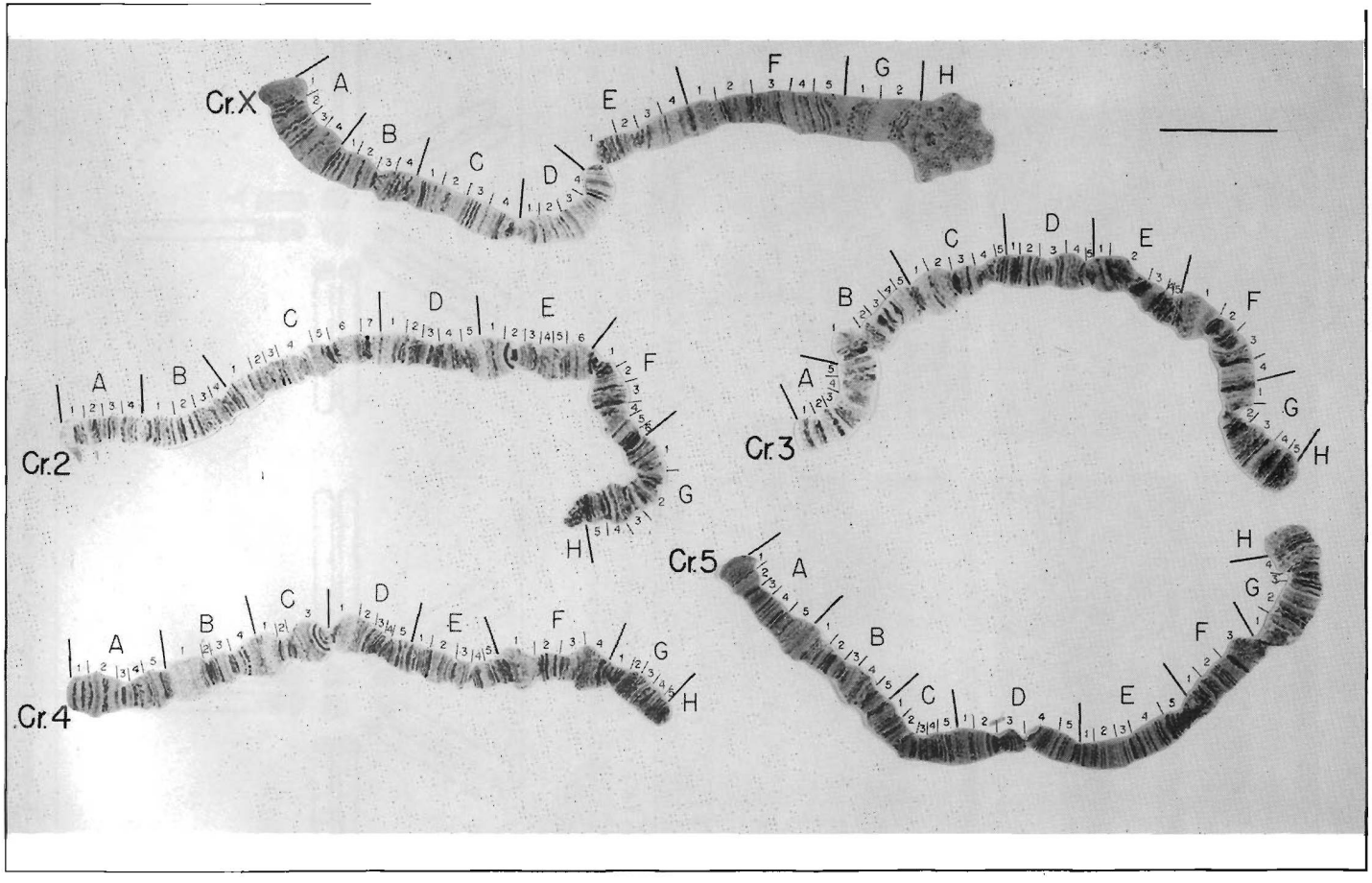

Figure 5 - Photographic salivary gland chromosomes map of *Drosophila eleonorae*, sp. n. Chromosome 6 was intentionally omitted. Bar = 30 μm.

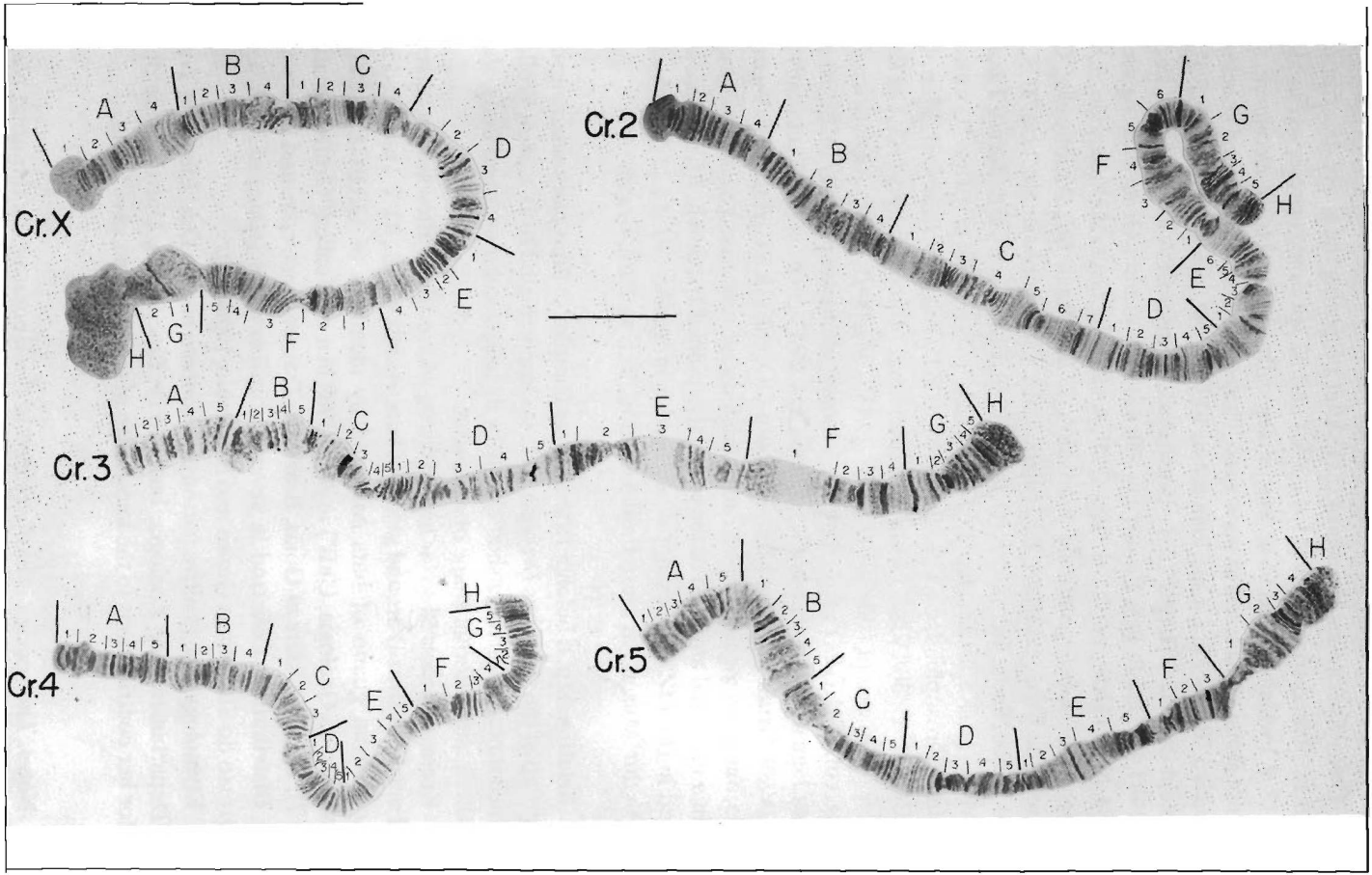

Figure 6 - Photographic salivary gland chromosomes map of *Drosophila repleta* Wollaston. Chromosome 6 was intentionally omitted. Bar = 30 μm.

Other Specimens Examined (51). After preparing the above description, we analyzed 18 pinned specimens (15 ♂, 3 ♀) and 33 specimens preserved in alcohol (16 ♂, 17 ♀) which proved to be morphologically identical to *D. eleonorae*, sp. n., but are not being considered as paratypes. They are as follows. (Pinned specimens): 3 ♂ labelled "Brasil Pará, Cav. Planaltina, 10.VII.1987, col. GEP, R.S. Paiva", 6 ♂ idem except date "12.VII.1987"; 5 ♂ "Brasil Pará, Transam. PA Km 80, Cav. Limoeiro, 18.VII.1987, col. GEP, R.S. Paiva"; 1 ♀ "Brasil MS, Bonito, Gruta N.S. Aparecida, 10.VII.84, N.M. Godoy col."; 1 ♂ "Brasil SP, Analândia, Gruta do Fazendão, 6.XI.83, E. Trajano col./sobre guano de morcego"; 2 ♀ "Brasil SP, Altinópolis, Gruta Sertãozinho de baixo, 25.III.84, E. Trajano col.". (Specimens in alcohol): 1 ♂, 1 ♀ "Gruta do Imbé, Padre Bernardo GO, 09.IX.1988, E. Trajano"; 1 ♂ "Gruta Tamboril, Unaí/MG, 10.XI.1988, E. Trajano col."; 1 ♀ idem plus "Guano de hematófago/Drosophilidae"; 1 ♀ "28/I/1989, 0069, Gruta do Tamboril, Mun. Unaí, GREGEO"; 2 ♀ "30/IV/89, Gruta de Jabuticaba, Mun. Formoso-GO, GREGEO 0084"; 1 ♀ idem except code "0107"; 2 ♂ "29/IV/1989, 0061, Gruta Jaguatirica, Mun. Formosa, col. GREGEO"; 1 ♂, 6 pupae, 9 larvae, idem except code "0128"; 10 ♂, 11 ♀ "Gruta Jaguatirica, Formosa, GO, Guano, col. 29.IV.1989, eclodido em cativeiro"; 1 ♂ "01/V/89, Gruta Paineira, Mun. Formosa-GO, col. GREGEO, 0133". Whenever available, one male specimen from each sampled cave was dissected and the genitalia placed in a microvial attached to the respective pinned specimen. All specimens are deposited in the MZUSP, except those bred from guano (10 ♂, 11 ♀) which are housed in the "Museu Paraense Emilio Goeldi", Belém, Pará, Brazil.

Relationship. It belongs to the *repleta* complex of the *repleta* subgroup of the *repleta* group of the subgenus *Drosophila*. It is closely related to *D. repleta* Wollaston from which it may be distinguished chiefly by its lighter color eyes and body, shorter spermatheca and different shape of aedeagus.

Ecology. Collected so far only in caves, where adults often swarm (mainly in caves of northern Brazil) around guano of bats. Adults (10 ♂, 11 ♀) emerged in the laboratory from guano of bats collected by members of GREGEO ("Grupo Espeleológico da Geologia, UnB") in April 1989 in a cave called "Gruta Jaguatirica" (Figure 7), Formosa, state of Goiás, Brazil (E. Trajano, pers. com. and label data).

Distribution. Collected in several brazilian caves (states of Pará, Goiás, Mato Grosso do Sul, Minas Gerais and São Paulo, Figure 7).

Etymology. The genitive patronym *eleonorae* honors Dra. Eleonora Trajano of the "Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo" for her contributions to the knowledge of the brazilian cave fauna.

Notes. The species is easily reared in the laboratory on the usual banana-brewer yeast media; however it does better on a media prepared with ground corn.

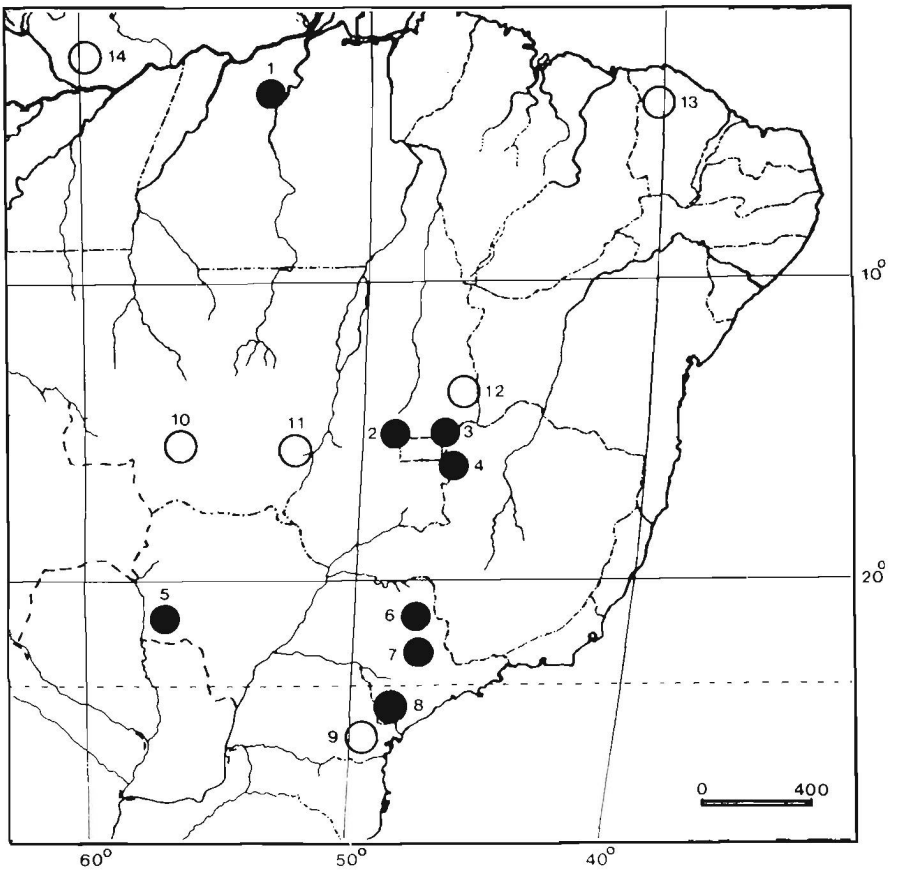

Figure 7 - Distribution of the known records of *Drosophila eleonorae*, sp. n. The solid circles (1-8) represent caves where the species was sampled; the open circles (9-14) represent some of the caves where no specimen was so far collected. the name cited after each number is of the toponym where the cave is to be found; the name of the caves are cited parenthetically. 1, Altamira (Limoeiro, Pedra da Cachoeira, Planaltina); 2, Padre Bernardo (Imbé); 3, Formosa (Jabuticaba, Jaguatirica, Paineira); 4, Unaí (Tamboril); 5, Bonito (Nossa Senhora Aparecida); 6, Altinópolis (Sertãozinho de baixo); 7, Ipeúna (Fazendão); 8, Iporanga (Bethary); 9, Rio Branco do Sul (Itacolombo); 10, Rosário d'Oeste (Currupira); 11, Chapada dos Guimarães (Kiogo Brado); 12, Conjunto São Mateus (Imbira); 13, Chapada de Ibiapaba (Ubajara); 14, Presidente Figueiredo (Maroaga). Based on examined specimens and after Godoy (1986), Trajano (1987) and Gnaspini-Netto (1989). Bar = 400 Km.

ACKNOWLEDGMENTS

We are deeply grateful to the G.E.P. ("Grupo de Espeleologia do Pará") to the GREGEO ("Grupo Espeleológico da Geologia UnB") and to the following speleologists for collecting and provid-

ing the specimens cited above: N.M. Godoy, R.S. Paiva and E. Trajano. We wish to thank "Produtos Alimentícios Fleischmann Royal Ltda." for providing Baker's yeast free of charge. This work was supported by CNPq/PIG (Proc. 403067/87), FAPESP (Proc. 87/0161-8) and USP.

RESUMO

Drosophila (*Drosophila*) *eleonorae*, uma nova espécie do grupo *repleta* habitante de cavernas, é descrita, ilustrada e comparada com a cosmopolita *D. repleta* Wollaston com a qual é relacionada. Apesar de morfologicamente distintas elas são homosequenciais com relação ao padrão de bandas dos cromossomos politênicos e têm idêntica distribuição de heterocromatina nos cromossomos metafásicos. Adultos de *D. eleonorae* sp. n. foram coletados em diversas cavernas localizadas em cinco estados do norte, centro e sudeste do Brasil (Pará, Goiás, Mato Grosso do Sul, Minas Gerais e São Paulo).

Vinte e um imagos emergiram, em laboratório, de guano de morcegos coletado em uma caverna do Brasil Central. Tentativas de produzir híbridos interespecíficos com *D. repleta* foram infrutíferas.

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(Received September 19, 1989)