PL-ISSN 0015-5497 (print), ISSN 1734-9168 (online)
Folia Biologica (Kraków), vol. 60 (2012), No 3-4
doi: 10.3409/fb60_3-4.129-133

Karyotype of the Bird Blowfly, *Protocalliphora falcozi* Séguy, 1928 (Diptera, Calliphoridae)*

Milada HOLECOVÁ, Maria ROŻEK, Anna MARYAŃSKA-NADACHOWSKA, and Viera JÁNOŠKOVÁ

Accepted May 22, 2012

HOLECOVÁ M., ROŻEK M., MARYAŃSKA-NADACHOWSKA A., JÁNOŠKOVÁ V. 2012. Karyotype of the bird blowfly, *Protocalliphora falcozi* Séguy, 1928 (Diptera, Calliphoridae). Folia Biologica (Kraków) **60**: 129-133.

The karyotype structure of *Protocalliphora falcozi* is described for the first time. The diploid complement comprises 2n=12, n♂=5+XY. Male mitotic plates include four pairs of long (metacentric) and one pair of medium-sized (submetacentric) autosomes. The submetacentric sex chromosomes X and Y are the smallest elements of the set. An achiasmatic meiosis was determined. The heterochromosomes do not form a heterovalent because they occur separately during the first meiotic division. The examined karyotype shows a pericentromeric position of constitutive heterochromatin in all autosomes. The longer arm of the X chromosome is heterochromatic, while the Y is entirely euchromatic. The NORs are active at mitotic prophase and at early meiotic stages. Small, bright signals were observed in the centromeric regions of autosomes after DAPI staining.

Key words: Diptera, Calliphoridae, *Protocalliphora falcozi*, karyotype, C-bands, NORs, fluorochrome staining.

Milada HOLECOVÁ, Department of Zoology, Comenius University, Mlynská dolina B-1, 842-15 Bratislava, Slovakia; Institute of Environmental and Regional Development, University of Central Europe in Skalica, Kráľovská 386/11, 909-01 Skalica, Slovakia.
E-mail: holecova@fns.uniba.sk
Maria ROŻEK, Anna MARYAŃSKA-NADACHOWSKA, Department of Experimental Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland.
E-mail: rozekms@wp.pl; maryanska@isez.pan.krakow.pl
Viera JÁNOŠKOVÁ, Department of Zoology, Comenius University, Mlynská dolina B-1, 842-15 Bratislava, Slovakia.
E-mail: viera.janoskova@gmail.com

Bird blowflies (*Protocalliphora* Hough) belong to the dipteran family of Calliphoridae (subfamily Chrysomyinae). Their larvae are blood sucking parasites living on altricial nestlings. Fifteen species of *Protocalliphora* have been recorded from the Palaearctic region, 10 from Europe, and 29 from the Nearctic region, including *P. rognesi* which has a Holarctic distribution (SCHUMANN 1986; KURAHASHI 1993; ROGNES 2004). Bird blowflies inhabit mainly temperate parts of both hemispheres up to the northern limit of trees (BOYES & SHEWELL 1975).

So far only 6 species of *Protocalliphora* from the Nearctic region have been karyologically studied using only conventional staining (BOYES 1961; BOYES & VAN BRINK 1965; BOYES & SHEWELL 1975). No data are known on the C-banding pattern and localization of NORs in karyotypes of bird blowflies.

Although considerable morphological variation has been found in the karyotypes of the species in the family Calliphoridae, the chromosome number is very stable at 2n=12 with five pairs of autosomes and a heteromorphic sex pair (STEVENS 1908; METZ 1916, 1922; KENEUKE 1924; BOYES & WILKES 1953; BOYES & VAN BRINK 1965; BOYES & SHEWELL 1975; BEDO 1991; PARISE-MALTEMPI & AVANCINI 2001).

Our study is a continuation of research focused on the taxonomy and biology of bird blowflies in Central Europe (JÁNOŠKOVÁ & ORSZÁGH 2009; JÁNOŠKOVÁ *et al.* 2010). Their larvae represent important hematophagous ectoparasites mostly of cavity nesting birds, namely of small passerines.

*Supported by VEGA, grant number 1/0124/09 and KEGA, grant number 007UKF-4/2012 to Milada HOLECOVA.

The higher prevalence of blowfly larvae in bird nests can aggravate health conditions and/or cause, in cooperation with other pathogens, an increase in nestling mortality (PUCHALA 2004).

In this paper, we examined the karyotype of *Protocalliphora falcozi* from Central Europe. In addition, we identified the constitutive heterochromatic regions and NORs in the chromosomes of this species using differential staining, namely C-banding, silver impregnation and fluorochromes DAPI and CMA_3.

Material and Methods

Sampling of the material was conducted in the National Nature Reserve Šúr situated in SW Slovakia on the SE fringe of the Malé Karpaty Mts. (48° 42' N, 17° 16' E). This conservation area is formed by rare alder wetlands in a flooded terrain depression combined with thermophilous oak woods. The material was sampled in May and June 2006, 2007 and 2011 from nests of the tree sparrow (*Passer montanus*). Some of the larvae were kept and allowed to develop into puparia and into adults, and this permitted species identification. Voucher specimens are deposited in the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków. Gonads from 20 freshly hatched males were dissected under a stereomicroscope in several drops of hypotonic 0.9 % sodium citrate solution containing 0.005 % colchicine. The gonads were transferred into a small volume of the same solution and incubated for 45 min at room temperature. Then the gonads were fixed according to the method described by ROŻEK (1994) with minor modification (ROŻEK & LACHOWSKA 2001). C-banding was performed using the procedure described by SUMNER (1972) with some modifications. In order to reveal C-heterochromatin, slides were treated with 45% acetic acid for 15 minutes, immersed in a saturated solution of $Ba(OH)_2$ at room temperature for 6 minutes, rinsed in distilled water, immersed in 2xSSC at 60°C for one hour, rinsed, air-dried, and stained with 4% solution of Giemsa in Sörensen's buffer (pH 6.8) for 10 to 20 min. Stained slides were rinsed with distilled water, air dried and mounted in Entellan. Nucleolar organizing regions (NORs) were stained after HOWELL & BLACK (1980) with slight modification (LACHOWSKA *et al.* 2005). Slides were treated in 50% $AgNO_3$ with 20% solution of gelatine as the developer in a moist chamber at 50°C for 25 min. CG-specific chromomycin A_3, and AT-specific 4'-6-diamidino-2-phenylindole (DAPI) were used as fluorochromes to reveal the molecular composition of C-heterochromatin according to the methods given by SCHWEITZER (1976) and DONLON & MAGENIS (1983). All chromosomal slides were examined using a Nikon Eclipse E400 fluorescence microscope, at 1000x. Microphotographs were taken with a Nikon DS-U1 camera. The images were optimized for best contrast and brightness by means of the Corel Photo-Paint 11 image-processing software. In order to facilitate the arrangements of karyograms, chromosome lengths were calculated as percentages of the total chromosome of the haploid set (% TCL), which also includes the sex chromosomes. Chromosomes were classified according to LEVAN *et al.* (1964).

Results and Discussion

The diplod complement of *P. falcozi* consists of 10 autosomes and the sex heterochromosomes X and Y. Male mitotic plates include four pairs of long (metacentric) and one pair of medium-sized (submetacentric) autosomes. A secondary constriction is visible on the 1st autosomal pair. The longest chromosomes include the 1st-4th pair of 23.12 %-16.60 % relative length, whereas the 5th pair accounts for 13.92 % of the total complement length.

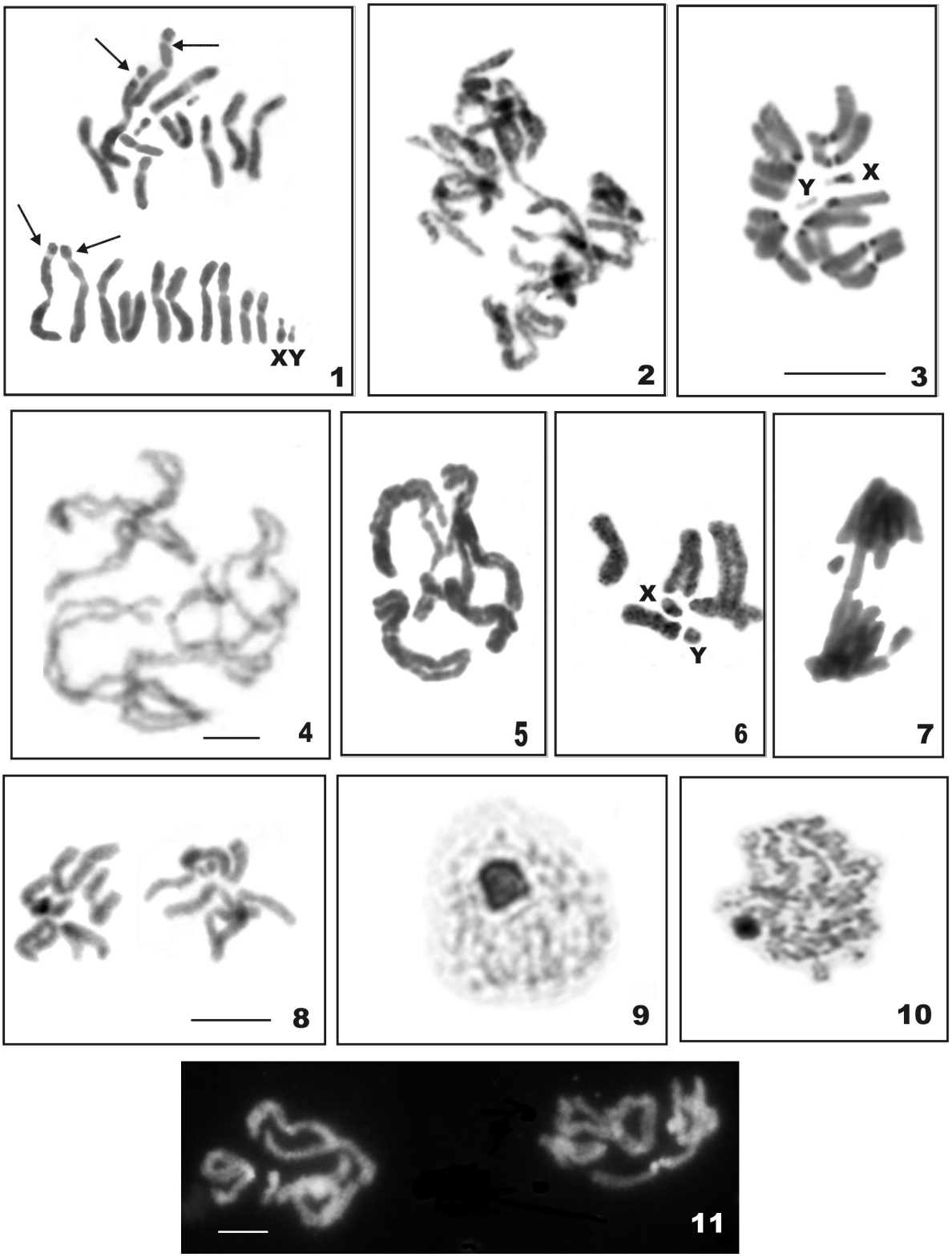
A heteromorphic bivalent is formed by submetacentric chromosomes X and Y with a relative length of 4.99 % and 3.54 %, respectively, of the entire karyotype (Table 1, Figs 1, 2, 3).

Examination of spermatocyte I cell divisions shows that meiosis is achiasmatic and there is no crossing-over because in diplotene to metaphase I the autosomal bivalents consist of two parallel homologues, separated from one another and there are no chiasmata. In metaphase I chromosomes X and Y always lay separate (Figs 4, 5, 6). As a result of anaphase I are two sister metaphases II with n = 5+X

Table 1

Total complement length (% TCL) and arm ratio (AR) of particular chromosome pairs in the karyotype of *Protocalliphora falcozi*. Abrreviations: M – metacentric, SM – submetacentric

Pair no.	1	2	3	4	5	X	Y
AR	1.22(M)	1.30(M)	1.33(M)	1.46(M)	1.80(SM)	1.92(SM)	2.30(SM)
% TCL*	23.12	19.84	17.99	16.60	13.92	4.99	3.54



Figs 1-11. Mitotic and meiotic chromosomes of the blowfly *Protocalliphora falcozi.*
Fig. 1. Mitosis, spermatogonial metaphase and karyogram, arrows indicate secondary constriction in the first pair of autosomes. Fig. 2. Mitotic anaphase. Fig. 3. C-banding mitotic metaphase. Fig. 4. Diplotene. Fig. 5. Diakinesis. Fig. 6. Metaphase I with separate X and Y chromosomes. Fig. 7. Anaphase I. Fig. 8. Two daughter metaphases II. Fig. 9. Leptotene/zygotene stained with $AgNO_3$. Fig. 10. Pachytene/diplotene, stained with $AgNO_3$. Fig. 11. DAPI-staining, metaphases II with positive signal in centromeres. Bars – 10 μm.

and n = 5+Y respectively All chromosomes show reductional segregation in the meiosis I (Figs 7, 8).

Heterochromatin visualized by C-banding was found in the pericentromeric region of all autosome C-blocks, limited to small centromeric bands as shown in early prophase (not illustrated) as well as in mitotic metaphase, whereas the X chromosome has a longer heterochromatic arm while the Y is entirely euchromatic (Fig. 3).

One nucleolar organizer region (NOR) impregnated with silver is visible in the mitotic prophase. Ag-positive NORs were not observed on chromosomes of the mitotic metaphase. Nevertheless, the morphology of the karyotype suggests that the secondary constriction present in the 1st pair of autosomes is associated with the nucleolar organizer region. One NOR is also recognizable from leptotene to diplotene. Argentophilic blocks are absent in later meiotic stages (Figs 9, 10).

Small, bright signals were observed in the centromeric regions of autosomes after DAPI staining, indicating that heterochromatin consists of repeats rich in A-T nucleotides (Fig. 11).

The chromosome complement of *P. falcozi* agrees with the earlier finding that members of the family Calliphoridae show remarkable karyotypic uniformity with 2n=12, comprising five pairs of larger or medium-sized meta/submetacentric autosomes and smaller XY sex chromosomes. The autosomes of Calliphoridae reveal a great deal of stability as compared to the sex chromosomes which show variation from one species to another in size and shape (BOYES & SHEWELL 1975; AZEREDO-ESPIN & PAVAN 1983; ULLERICH & SCHOTTKE 2006). The autosomes of *P. falcozi* show very close side-by-side pairing (somatic pairing) of homologues, a characteristic feature of the chromosome complement of all the dipterans in which homologous chromosomes tend to lie next to one another. As a result of the somatic pairing, the diploid complements sometimes give the appearance of a haploid set (AGRAWAL *et al.* 2010). However, the sex chromosomes do not show such intimate somatic pairing and tend to lie separately (BOYES & SHEWELL 1975; ULLERICH & SCHOTTKE 2006; AGRAWAL *et al.* 2009, 2010, and the present study). Male meiosis in *P. falcozi* described in this paper compared with earlier results lead to the conclusion that achiasmatic spermatogenesis is the rule in the family Calliphoridae (ULLERICH 1976, 1980, 1984, 1996). The quantity and localization of constitutive heterochromatin have a significant role in sex chromosome evolution. The incorporation of heterochromatin represents an initial step in the speciation of these chromosomes (JOHN 1988; JABLONKA & LAMB 1990). Most dipteran species have heterochromatin which can extend from the pericentromeric region to include whole arms of some chromosomes or even the entire sex chromosomes (BEDO 1991). BOYES & VAN BRINK (1965) showed a tendency for the X chromosome (and to a lesser extent the Y) to accumulate heterochromatin in several subfamilies of calyptrate Diptera. The loss and/or gain of heterochromatin in the sex chromosomes has played a great role in karyotypic evolution in Calliphoridae as suggested by BOYES & VAN BRING (1965).

NORs are also good markers for evolutionary studies since the rDNA genes are extremely well conserved among dipteran species. In most Diptera, the NORs are located in the sex chromosomes (BEDO & HOWELLS 1987). In the present study, the NOR is probably located on the first pair of autosomes at the site of the secondary constriction and not in the sex chromosomes. This situation may represent an intermediate step in the chromosomal evolution of this group. Some genome sequences, including the major ribosomal genes, may have moved from the sex chromosomes to autosomes, in order to avoid damage due to a partial loss of the sex chromosomes (PARISE-MALTEMPI & AVANCINI 2007).

Chromosome staining by DNA base-specific fluorochromes is little used in cytogenetic studies of Diptera and has never hitherto been applied to *Protocalliphora*. The use of fluorescent DNA banding dyes with different specificities better characterizes heterochromatic regions in terms of their relative enrichment with A-T or G-C base pairs. In *P. falcozi*, a bright signal after DAPI staining suggests the occurrence of a high amount of A-T base pairs in the DNA sequences making up the heterochromatic C-bands. Some differences in fluorescent intensity could be explained by the degree of condensation, i.e. the more the chromosomes are elongated, the weaker the visible signals. The sequential CMA_3 staining of chromosomes of *P. falcozi* showed that heterochromatin is negatively stained by chromomycine which supports the hypothesis that there is an abundance of A-T in heterochromatin. The fluorochrome CMA_3 labels NORs independently of their activity, and the fluorescence is associated with G-C content typical of genes coding for ribosomal RNA (rDNA) (ANOKHIN & NOKKALA 2004). The correlation between CMA_3 bands and NORs is quite common in insects (BRITO *et al*. 2003). The lack of positive signals in the examined species may suggest a small number of rDNA genes; alternatively, the absence of CMA_3 bands may be due to technical reasons because sometimes this band disappears when C-banding is applied before sequential staining with chromomycine (BRITO *et al.* 2003).

References

AGRAWAL U. R., BAJPAI N., KURAHASHI H., TEWARI R. R. 2009. Spontaneous triploidy in the blow-fly *Aldricina grahami* (Aldrich) (Calliphoridae: Diptera). Chromosome Science **12**: 35-57.

AGRAWAL U. R., BAJPAI N., KURAHASHI H., TEWARI R. R. 2010. Metaphase karyotypes of four species of Calliphoridae (Diptera). Chromosome Science **13**: 49-52.

ANOKHIN B., NOKKALA S. 2004. Characterization of C-heterochromatin in four species of Hydrozoa (Cnidaria) by sequence specific fluorochromes Chromomycin A_3 and DAPI. Caryologia **57**: 163-166.

AZEREDO-ESPIN A. M. L., PAVAN C. 1983. Karyotypes and possible regions of origin of three species of Calliphoridae (Diptera) recently introduced in Brazil. Rev. Brasil. Genet. **4**: 619-638.

BEDO D. G. 1991. Cytological characterization of heterochromatin in mitotic and meiotic chromosomes of the old screw worm fly *Chrysomya bezziana* (Diptera: Calliphoridae). Genome **34**: 631-637.

BEDO D. G., HOWELLS A. T. 1987. Chromosomal localization of the white gene of *Lucilia cuprina* (Diptera: Calliphoridae) by in situ hybridization. Genome **29**: 72-75.

BOYES J. W. 1961. Somatic chromosomes of higher Diptera. V. Interspecific and intraspecific variation in the Calliphoridae. Can. J. Zool. **39**: 549-570.

BOYES J. W., VAN BRINK J. M. 1965. Chromosomes of calyptrate Diptera. Can. J. Genet. Cytol. **7**: 537-550.

BOYES J. W., SHEWELL G. E. 1975. Cytotaxonomy of Calliphoridae (Diptera). Genetica **45**: 435-488.

BOYES J. W., WILKES A. 1953. Somatic chromosomes of higher Diptera I. Differentiation of tachinid parasites. Can. J. Zool. **31**: 125-165.

BRITO R. M., CAIXERIO A. P., POMPOLO S. G., AZEVEDO G. G. 2003. Cytogenetic data of *Portamona peckoli* (Hymenoptera, Apidae, Meliponini) by C banding and fluorochrome staining with DaA/CMA_3 and DA/DAPI. Gen. Mol. Biol. **26**: 53-57.

DONLON T. A., MAGENIS R. E. 1983. Methyl green is a substitute for distamycin A in the formation of distamycinA/DAPI C-bands. Hum. Genet **65**: 144-146.

HOWELL W., BLACK D. A. 1980. Controlled silver-staining of nucleolus organizer regions withprotective colloidal developer: a 1-step method. Experientia **36**: 1014-1015.

JABLONKA E., LAMB M. I. 1990. The evolution of heteromorphic sex chromosomes. Biol. Rev. **65**: 249-279.

JÁNOŠKOVÁ V., ORSZÁGH I. 2009. Synchronization between bird blowfly ontogeny and nestling ontogeny in State Nature Reserve Šúr. Comenius University, Bratislava, 90 pp . (In Slovak with English summary).

JÁNOŠKOVÁ V., ORSZÁGH I., JAMRIŠKA J., KOPÁNI M. 2010. Pre-imaginal stages of the blowfly *Protocalliphora falcozi* in nests of the tree sparrow (*Passer montanus*). Entomol. Fennica **21**: 107-116.

JOHN B. 1988. The biology of heterochromatin. (In: Heterochromatin: Molecular and Structural Aspects. R. S. Verma ed. Cambridge University Press, Cambridge): 1-147.

KENEUKE W. 1924. Über die Spermatogenese eigener Dipteren. Z. Zell. Gewebelehre **1**: 357-412.

KURAHASHI H. 1993. Japanese species of the ornithoparasitic blow flies: *Protocalliphora* and *Trypocalliphora* (Diptera: Calliphoridae). New Entomologist **42**: 8-15.

LACHOWSKA D., HOLECOVÁ M., ROŻEK M. 2005. C-banding karyotype and NORs analyse in eight species of *Barypeithes* Duval from Central Europe (Coleoptera, Curculionidae, Entiminae). Caryologia **58**: 274-280.

LEVAN A., FREDGA K., SONBERG A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201-220.

METZ C. W. 1916. Chromosome studies on Diptera. II. The paired association of chromosomes in Diptera and its significance. J. Exp. Zool. **21**: 213-280.

METZ C. W. 1922. Association of homologous chromosomes in tetraploid cells of Diptera. Biol. Bull. **43**: 369-373.

PARISE-MALTEMPI P. P., AVANCINI R. M. P. 2001. C-banding and FISH in chromosomes of the blow flies *Chrysomya megacephala* and *Chrysomya putoria* (Diptera, Calliphoridae). Mem. Inst. Oswaldo Cruz, Rio de Janeiro **96**: 371-377.

PARISE-MALTEMPI P. P., AVANCINI R. M. P. 2007. Comparative cytogenetic study in Muscidae flies. Braz. J. Biol. **67**: 945-950.

PUCHALA P. 2004. Debrimental effects of larval blow flies *Protocalliphora azurea* on nestlings and breeding success of Tree Sparrows (*Passer montanus*). Can. J. Zool. **82**: 1285-1290.

ROGNES K. 2004. *Protocalliphora*. (In: Fauna Europea: Calliphoridae. File Online.Version 2.4. http://www.faunaeur.org/ T. Pape ed. 27. January 2011).

ROŻEK M. 1994. A new chromosome preparation technique for Coleoptera (Insecta). Chromosome Research **2**: 76-78.

ROŻEK M., LACHOWSKA D. 2001. C-bands on chromosomes of four beetle species (Coleoptera: Carabidae, Silphidae, Elateridae, Scarabaeidae). Folia Biol. (Kraków) **49**: 179-182.

SCHUMANN H. 1986. Family Calliphoridae. (In: Catalogue of Palaearctic Diptera. vol. 12. A. Soós & L. Papp eds. Akadémiai Kiádó, Budapest): 11-58.

SCHWEITZER D. 1976. Reverse fluorescent chromosome banding with chromomycin and DAPI. Chromosoma **58**: 307-324.

STEVENS N. M. 1908. A study of the germ cells of certain Diptera with special reference to the heterochromosomes and the phenomenon of synapsis. J. Exp. Zool. **5**: 359-374.

SUMNER A. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell. Res. **75**: 304-306.

ULERICH F.-H. 1976. Chromosomenverhältnisse, konstitutives Heterochromatin und Geschlechtsbestimmung bei einigen Arten der Gattung *Chrysomya* (Calliphoridae, Diptera). Chromosoma **58**: 113-136.

ULLERICH F.-H. 1980. Analysis of the predetermining effect of a sex realizer by ovary transplantation in the monogenic fly *Chrysomya rufifacies.* Wilhelm Roux´ Arch. **188**: 37-43.

ULLERICH F.-H. 1984. Analysis of sex determination in the monogenic blowfly *Chrysomya rufifacies* by pole cell transplantation. Mol. Gen. Genet. **193**: 479-487

ULERICH F.-H. 1996. Inheritance patterns of new genetic markers snd occurrence of spontaneous mosaicism in the monogenic blowfly *Chrysomya rufifacies* (Diptera: Calliphoridae). Mol. Gen. Genet. **253**: 232-241.

ULLERICH F. H., SCHOTTKE M. 2006. Karyotypes, constitutive heterochronmatin and genomic DNA values in the blowfly genera *Chrysomya*, *Lucilia* and *Protophormia* (Diptera: Calliphoridae). Genome **49**: 584-597.