Cytogenetic Features of Human Head and Body Lice (Phthiraptera: Pediculidae)

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ABSTRACT The genus *Pediculus* L. that parasitize humans comprise two subspecies: the head lice *Pediculus humanus capitis* De Geer and the body lice *Pediculus humanus humanus* De Geer. Despite the 200 yr of the first description of these two species, there is still a long debate about their taxonomic status. Some authors proposed that these organisms are separate species, conspecifics, or grouped in clades. The sequencing of both forms indicated that the difference between them is one gene absent in the head louse. However, their chromosomal number remains to be determined. In this study, we described the male and female karyotypes, and male meiosis of head and body lice, and examined the chromatin structure by means of C-banding. In *P. h. humanus* and *P. h. capitis*, the diploid chromosome complement was $2n = 12$ in both sexes. In oogonial prometaphase and metaphase and spermatogonial metaphase, it is evident that chromosomes lack of a primary constriction. No identifiable sex chromosomes or B chromosomes were observed in head and body lice. Neither chiasmata nor chromatin connections between homologous chromosomes were detected in male meiosis. The meiotic behaviour of the chromosomes showed that they are holokinetic. C-banding revealed the absence of constitutive heterochromatin. Our results provide relevant information to be used in mapping studies of genes associated with sex determination and environmental sensing and response.

KEY WORDS *Pediculus humanus capitis*, *Pediculus humanus humanus*, holokinetic chromosome, karyotype, C-banding

The monophyletic family Pediculidae is composed of only the genus *Pediculus* L., 1758 with four species plus three *nomina nuda*, which are found on humans and other primates (Kim and Ludwig 1978, Durden and Musser 1994, Castro and Cicchino 1998, Gillot 2005). In 1758, Linnaeus first described the lice of humans as *Pediculus humanus* and then described the lice from the hair and from the clothes as different varieties (Kim and Ludwig 1978).

The human head and body lice have infested humans for the 5–6 million yr ago when the ancestors of chimpanzees and humans diverged (Pennísí 2004). Both lice are strict and obligate human ectoparasites that differ mainly in their habitat on the host. Head lice live and feed exclusively on the scalp, and the female attach their eggs to the base of hair shafts of the scalp, whereas body lice feed in the body region of humans and lay eggs on clothing (Buxton 1946, Burgess 2004). There are strong evidences supporting the theory that body lice descended from head lice, when humans lost their body hair and started wearing clothes (Reed et al. 2004).

Despite these habitat differences, the taxonomic status of the head and body lice remains uncertain. Until recently, *Pediculus capitis* De Geer and *Pediculus humanus* L. were considered sibling species (Gillot 2005). In fact, both species might interbreed readily and produce viable offspring under certain conditions at the laboratory (Levene and Dobzhansky 1959). For this reason, they are recognized as subspecies of *P. humanus*, *P. humanus capitis* De Geer and *Pediculus humanus corporis* De Geer or *P. humanus humanus* L. (Durden and Musser 1994). However, in nature (i.e., on the host), they retain their morphometric identities, are kept separate genetically by ecological or behavioural factors, and do not interbreed. This has led to treat them as separate species (Schaefer 1978). This author proposed that specimens of both *P. capitis* and *P. humanus* may be considered as separate species because on a single host, they are not only spatially isolated (allopatric) but also ecologically isolated.

In addition, there are strong differences in their vector capacity. Head lice are known to infest millions of school children worldwide with symptoms like itching, social stigma, and loss of sleep; however, no human pathogens transmission has been reported (Light et al. 2008). In contrast, body lice are known to transmit three bacteria pathogens such as *Borrelia recurrentis* (louse-borne relapsing fever), *Bartonela quintana*

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(trench fever), and *Rickettsia prowazekii* (epidemic typhus) (Mumcuoglu et al. 2009).

On the other hand, molecular studies of mitochondrial and nuclear DNA indicated that body and head lice are conspecific (i.e., belong to the same species), despite their differences in morphology, size, and habitats on the host (Veracx et al. 2012). Additionally, analysis of the transcriptome of the head louse revealed that this organism had almost the exact same numbers of genes as the body louse, with only one gene "missing" in the head louse (Olds et al. 2012; Drali et al. 2013).

Cytogenetic studies carried out in 15 species of the order Phthiraptera revealed the following features: a particular spermatogenesis, presence of holokinetic chromosomes (i.e., chromosomes without a primary constriction and thus without a localized centromere), a low diploid chromosome number ($2n = 4$–24), an absence of identifiable sex chromosomes, and an achiasmatic male meiosis (Cannon 1922, Sharma and Malik 1953, Bayreuther 1955, Scholl 1955, Tombesi and Papeschi 1993, Tombesi et al. 1999, Golub and Nokkala 2004). Spermatogenesis differs from those found in other insects and follows a peculiar course. After first meiotic division, each cell divides mitotically and gives rise to a cyst of 32/64 active spermatozoa and 32/64 nonfunctional cells (Hindle and Pontecorvo 1942, Sharma and Malik 1953, Bayreuther 1955, Scholl 1955, Tombesi and Papeschi 1993, Tombesi et al. 1999, Golub and Nokkala 2004). The number of functional and nonfunctional cells in a cyst depends on the species, and could be considered as species specific character (Tombesi and Papeschi 1993). In the genus *Pediculus*, the cytogenetic studies from the beginning of the past century were concerned with the male diploid and/or haploid chromosome numbers of *Pediculus capitis* ($2n = 12$; $n = 6$), *P. corporis* ($2n = 12$; $n = 6$) and *Pediculus vestimenti* Nitzch, 1818 ($2n = 10$; $n = 5$; Tombesi and Papeschi 1993). As mentioned by Pittendrigh et al. (2006), the actual haploid chromosome number of either head or body louse remains to be determined.

In the present work, we analyzed the male and female karyotypes and male meiosis of two human lice, *P. h. capitis* and *P. h. humanus*. We also examined the chromatin structure by means of C-banding to find distinct cytogenetic features to elucidate their taxonomic status. Our results provide relevant information to be used in mapping studies of genes associated with sex determination and environmental sensing and response.

Materials and Methods

Lice. Head lice specimens were collected from heads of children aged between 5 and 12 years old from one primary school of Buenos Aires, Argentina. For the study at the schools, permission to perform the study was obtained from the educational authority of the Government of Buenos Aires city. All the studied schools were Argentinean Government owned and nonfee-paying. Only pupils whose parents had given informed consent for participation were examined. The freedom to refuse to participate in the research was clearly and amply established in each case. The entire head was examined carefully, although special attention was paid to the nape of the head and behind the ears. The scalp was examined for a period of 5 min. Head lice were removed using a fine-toothed antilouse metal comb, and were collected and transported to our laboratory, as previously reported by Picollo et al. (1998). The protocol for lice collection was approved by the ad hoc committee of the Centro de Investigaciones en Plagas e Insecticidas (Research Center of Pests and Insecticides, Buenos Aires, Argentina), and archived in our laboratory.

The body lice were obtained from the colony maintained for 9 yr at our laboratory. This colony was originated from the Orlando strain of *P. h. humanus* at the University of Queensland, Brisbane, Australia. The colony was maintained at $28 \pm 1°C$, $55 \pm 1\%$ relative humidity (RH), and was fed on rabbits six times per week for 20–30 min.

Chromosome Preparations. The specimens were brought alive to the laboratory (Table 1). The gonads of male and female nymphs (II and III) and adults of both *P. h. capitis* and *P. h. humanus* were dissected in physiological solution for *Ephestia* (Glaser 1917 cited in Lockwood 1961) and fixed for 30 min in freshly prepared fixative solution (ethanol: acetic acid, 3:1) for classical cytogenetic analysis. Slides were performed by the squash technique in a drop of 2% iron acetic hematoxylin following conventional procedures (Sáez 1960). After being squashed, the preparations were sealed with nail varnish. For DAPI-staining and C-banding, the gonads were dissected in physiological solution, swollen for 10 min in a hypotonic solution (0.075 M KCl), and then fixed in freshly prepared Carnoy fixative (ethanol: chloroform: acetic acid, 6:3:1). Chromosome preparations were made by spreading cells in a drop of 60% acetic acid with tungsten needles and spread on the slide using a heating plate at 45°C (Traut 1976). Then, the preparations were dehydrated in an ethanol series of 70, 80, and 96% (30 s each), air dried, and stored at −20°C for DAPI-staining or C-banding.

DAPI-staining and C-banding. DAPI-staining was performed according to Traut (1999) and Sahara et al. (1999). C-banding was applied to spread chromosome preparations to reveal constitutive heterochromatin. C-banding was performed according to Papeschi (1988) and Poggio et al. (2011). Briefly, slides were treated with 0.2 N HCl at 60°C for 1 min, immersed in a saturated solution of $Ba(OH)_2$ at room temperature for 15 min, thoroughly rinsed in water and then immersed in 2× SSC at 60°C for 1 h (Papeschi 1988).

Table 1. Number of specimens analyzed of *P. h. humanus* and *P. h. capitis*

	Nymph		Adult		
	Female	Male	Female	Male	Total
P. humanus humanus	2	9	3	4	18
P. humanus capitis	2	–	1	11	14

The pretreated slides were stained with 4'6-diamidino-2-phenylindole (DAPI; Fluka BioChemika, Sigma Aldrich Production GmbH, Buchs, Switzerland) for a better resolution of C-bands (Poggio et al. 2011). It has been shown that the C-DAPI banding technique revealed the same heterochromatic regions that C-Giemsa banding (Barros e Silva and Guerra 2010).

Microscopy and Image Processing. Preparations were observed in a Leica DMLB microscope equipped with a Leica DFC350 FX CCD camera and the Leica IM50 version 4.0 software (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). After examination all chromosome preparations, typical cells from each best chromosome preparations were captured by the digital camera. Black-and-white images of chromosomes were recorded. Images were pseudocolored (light blue for DAPI) and processed with Photoshop (CS6; AdobeSystems, Mountain View, CA, Photoshop).

Results

Chromosome Complement and Meiosis. In *P. h. humanus* and *P. h. capitis*, the diploid chromosome complement is $2n = 12$ in both sexes (Fig. 1; Table 1). In oogonial prometaphase (Fig. 1a and b) and metaphase (Fig. 1c) and spermatogonial metaphase (Fig. 1d), chromosomes lack of a primary constriction and one pair is slightly larger (Fig. 1a and b). At oogonial (Fig. 1c) and spermatogonial metaphases (Fig. 1d), the chromosomes show the sister chromatids separated from each other by a regular distance. In oogonial prometaphase, an association between a nucleolus and two chromosome pairs is also observed (Fig. 1a and b). In male mitotic metaphase, sister chromatids are disposed parallel to each other (Fig. 1d), and at anaphase they migrate parallel to each other, but the telomeres seem to precede migration (Fig. 1e). This kind of migration results in the formation of duplicated images of anaphase plates.

Since female meiosis is detained prior to the first meiotic division, i.e., at early meiotic prophase, it is not possible to describe all the meiotic stages. In *P. h. humanus* and *P. h. capitis*, male meiotic behavior is similar and follows the same pattern as previously described for other phthirapteran lice. Thus, combined meiotic stages from both species are shown in Figs. 1 and 2. During spermatogenesis, no cells at leptotene or zygotene are recognized, and in the few cells observed at pachytene six bivalents are clearly distinguished with different degrees of condensation (Fig. 1f). Neither typical diplotene or diakinesis stages nor heteromorphic sex chromosomes are detected. At metaphase I, six achiasmatic bivalents are clearly identified and gradually condensed without modifying their morphology, whereas the nucleolus disappears. During this stage, the bivalents dispose forming a circle and one of them usually lies at the centre of it (Fig. 1g and h). It becomes evident that no chiasma is present and homologue chromosomes lie side by side. However, in some autosomal bivalents chromatin connections between homologues can sometimes be detected, which are nonchiasmatic in origin. Thus, it is possible to observe six no chiasmata pair of chromosomes. At anaphase I, the sister chromatids of each chromosome separated to opposite poles, i.e., equational segregation (data not shown). After telophase I (Fig. 1i), the second meiotic division takes place (Fig. 1j). The resulting haploid cells, i.e., primary spermatids, divide mitotically in perfect synchronization and give rise to cysts of 32 cells, i.e., secondary spermatids (Fig. 2a and b). Then, these cells go through a last mitosis, originating a cyst of 32 tertiary spermatids and 32 pycnotic nuclei (Fig. 2c). The latter degenerate and the tertiary spermatids develop into 32 active spermatozoa in each bundle (Fig. 2d).

C-banding. There are no heterochromatic differences between head and body lice by the C-banding technique (Fig. 3). During interphase, only the nucleolus and small C-positive dots are observed (Fig. 3a), but their presence can no longer be detected from pachytene onward (Fig. 3b and c).

Discussion

In the order Phthiraptera, only few cytogenetic studies have been carried out, and only the karyotype and the chromosome behaviour of 15 species have been described (Foot 1919, Doncaster and Cannon 1920, Cannon 1922, Ries 1932, Perrot 1934, Hindle and Pontecorvo 1942, Sharma and Malik 1953, Bayreuther 1955, Scholl 1955, Tombesi and Papeschi 1993, Tombesi et al. 1999, Emeljanov et al. 2001, Golub and Nokkala 2004). However, many chromosome characteristics of Phthirapteran lice have been controversial. In the family Pediculidae, the last cytogenetic description of the chromosomal complement was performed more than 60 years ago in *P. corporis* (Hindle and Pontecorvo 1942). These authors reported that the somatic cells of both sexes, and also the oogonial and spermatogonia, had five metacentric chromosomes and one telocentric chromosome ($2n = 12$), indicating the presence of a localized centromere. Recently, Veracx and Raoult (2012) reviewed the biology and genetics of the human head and body lice, and determined that either species had six monocentric chromosomes. Nevertheless, all the previous studied lice of birds and mammals (excluding human lice) displayed holocentric chromosomes, i.e., without a primary constriction and, thus, without a localized centromere (Cannon 1922, Ries 1932, Perrot 1934, Bayreuther 1955, Scholl 1955, Tombesi and Papeschi 1993, Tombesi et al. 1999, Emeljanov et al. 2001, Golub and Nokkala 2004). Holokinetic chromosomes differ from monocentric chromosomes in many peculiar karyological features. In monocentric chromosomes, localized centromeres are observed at metaphase as partially decondensed chromosome regions, forming a primary constriction, typically flanked by heterochromatin (Morris and Moazed 2007). Contrarily, holokinetic chromosomes are homogenously condensed having neither primary constrictions nor heterochromatin-rich pericentromere organization (Guerra et al. 2010). Besides, holokinetic chromosomes also display a characteristic behavior at

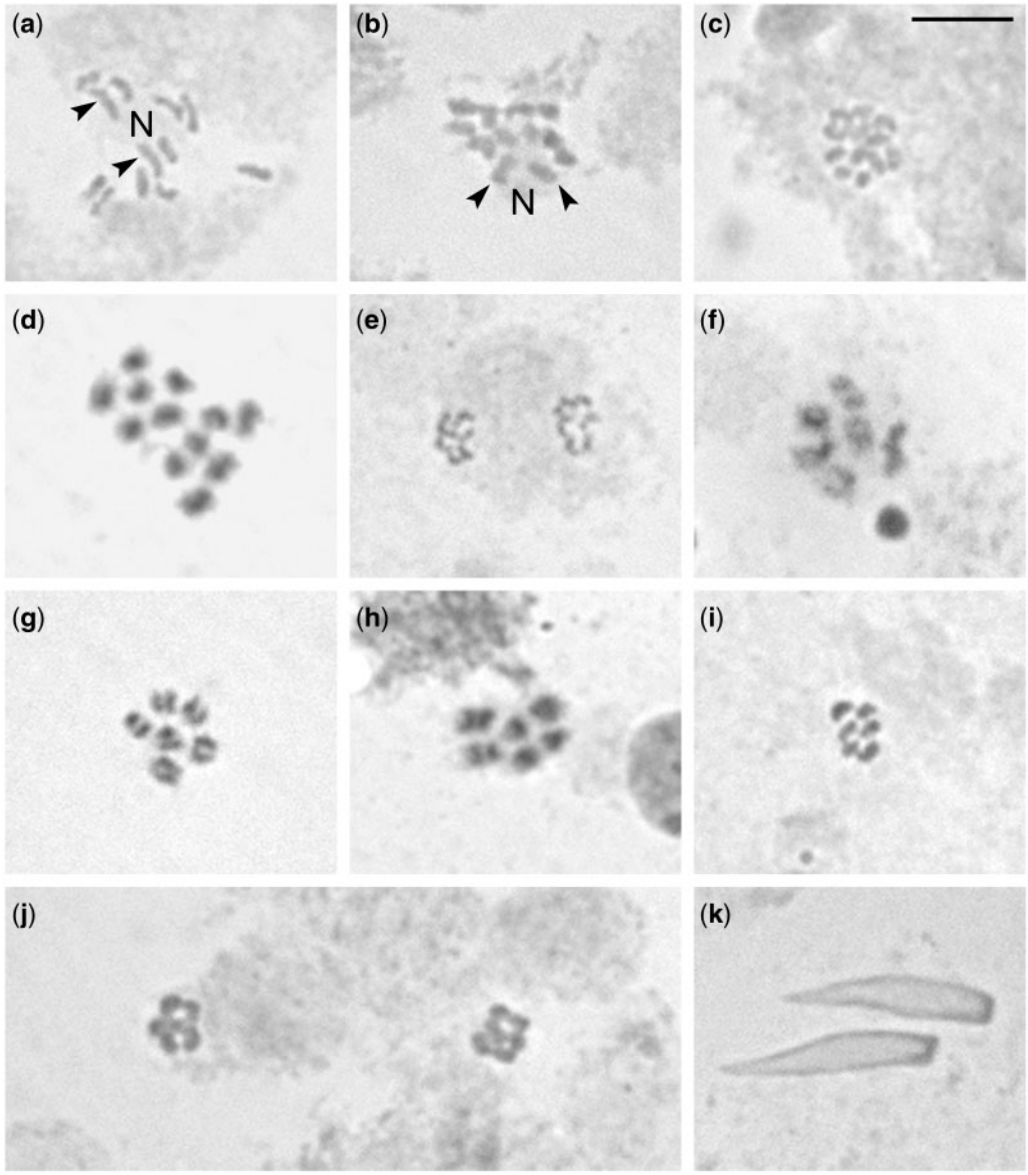

Fig. 1. Chromosome complement and meiosis in *P. h. humanus* (a–g, i–k) and *P. h. capitis* (h). (a and b) Oogonial prometaphase, arrowheads point one pair slightly larger. (c) Oogonial metaphase. (d) Spermatogonial metaphase. (e) Male mitotic anaphase. (f) Pachytene. (g) Metaphase I. (h) Metaphase I. (i) Telophase I. (j) Metaphase II. (k) Spermatids. N = nucleolus. Chromosomes are stained with 2% iron acetic haematoxylin. Bar = 10 µm.

mitosis, having their sister chromatids oriented parallel to the equatorial plate at metaphase and moving to the opposite poles parallel to each other during anaphase (Guerra et al. 2006, Mola and Papeschi 2006, Melters et al. 2012). Based on this background, our observations in *P. h. capitis* and *P. h. humanus* allow us to confirm the holokinetic nature of their chromosomes and, also, the female and male karyotype of both human lice by DAPI-staining and C-banding.

The earliest work on the spermatogenesis of human lice was done by (Foot 1919). According to this author, the number of chromosomes in the somatic as well as in the spermatogonial cells of *P. vestimenti* was 10 small chromosomes. The author also noticed an "unequal bivalent" in the primary spermatocytes, referring to this bivalent as the sexual chromosomes XY. Further reports showed that the somatic number of chromosomes of *P. corporis* and *P. capitis* was 12 but the spermatogonial division stages showed only six (Doncaster and Cannon 1920, Cannon 1922, Sharma and Malik 1953). Our analysis of spermatogonial and oogonial meiotic cells of *P. h. capitis* and *P. h. humanus* revealed that both human lice share the diploid chromosome number $2n = 12$. Besides, there

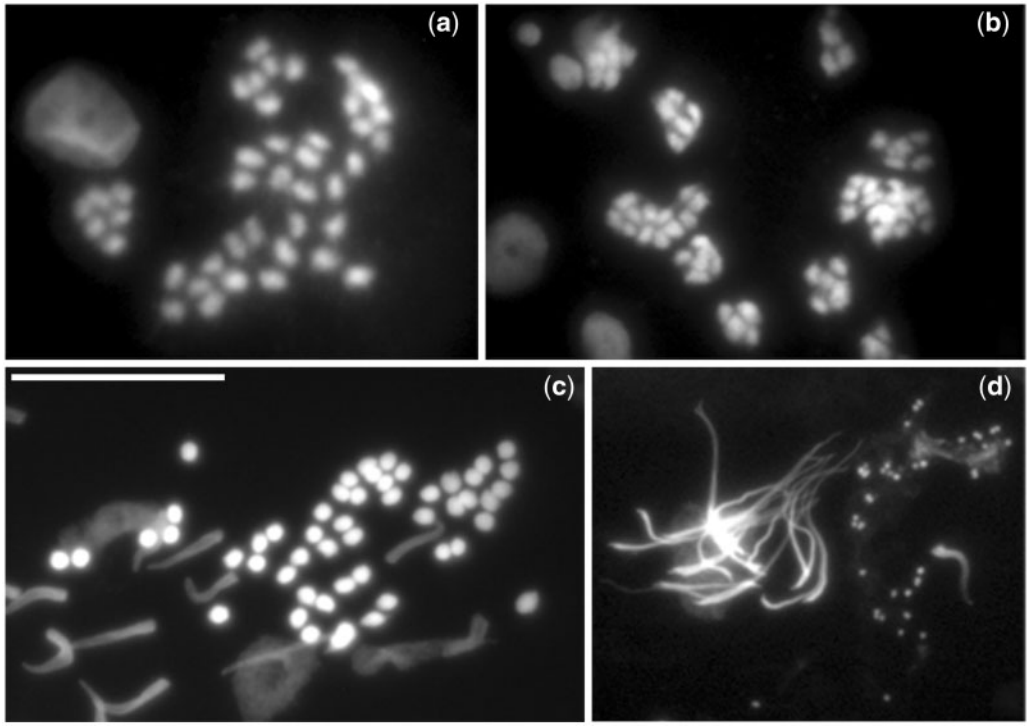

Fig. 2. Chromosome complement and meiosis in *P. h. humanus* (a) and *P. h. capitis* (b–d). (a–b) Secondary spermatids. (c) Pycnotic nuclei. (d) Cyst of 32 spermatozoa and 32 pycnotic nuclei. Chromosomes are stained with DAPI. Bar = 10 μm.

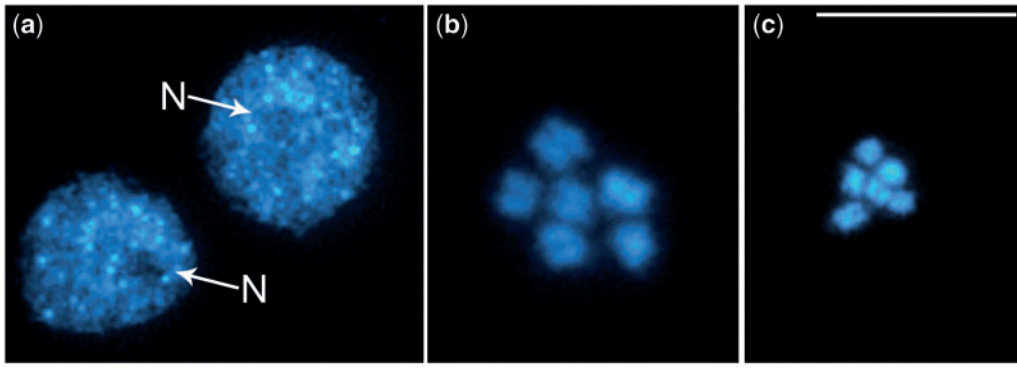

Fig. 3. C-banding followed by staining with DAPI in *P. h. humanus* (a) and *P. h. capitis* (b–c). (a) Interphase. (b) Metaphase I. (c) Metaphase II. N = nucleolus. Bar = 10 μm.

were neither clearly differences among the chromosomes nor the C-banding pattern in their karyotypes. No heteromorphic sex chromosome pair was identified because of the absence of chromosomal differences in size, staining, banding, or meiotic behavior in male lice specimens. This is in accordance to previous works on Phthiraptera where sex chromosomes were not possible to be identified (Tombesi and Papeschi 1993, Tombesi et al. 1999, Golub and Nokkala 2004). Nonetheless, the only reported case where a bivalent behaved slightly differently at metaphase I was found in *Haematopinus suis* (pig louse) (Tombesi and Papeschi 1993).

Spermatogenesis in Phthiraptera differs from most insect orders and follows a peculiar pattern: the four spermatids resulting from meiosis of one primary spermatocyte divide mitotically four or five times to give rise to a cyst of 32/64 cells (secondary spermatids). The last mitosis is unequal for the cytoplasm and results in a cyst of 32/64 tertiary spermatids and 32/64 nonfunctional cells, which will differentiate into active spermatozoa and pycnotic nuclei, respectively (Hindle and Pontecorvo 1942, Sharma and Malik 1953, Bayreuther 1955, Scholl 1955). The results in *P. h. capitis* and *P. h. humanus* of the present work are consistent with previous findings, i.e., each cell entering meiosis

experiences several mitosis in addition to the two meiotic divisions.

Another interesting feature in all phthirapteran species is the absence of chiasmata, at least in the male sex (Bayreuther 1955, Scholl 1955, Tombesi and Papeschi 1993, Tombesi et al. 1999, Golub and Nokkala 2004). A genetic consequence of the achiasmatic male meiosis in *P. h. capitis* and *P. h. humanus* is the absence of intrachromosomal recombination. This implies that the allelic combinations present in each chromosome of the males of both human lice will remain together and unchanged (except for mutation events) from one generation to another (Ituarte and Papeschi 2004). Because we have no information about the chiasma frequency in the females of these species, the low diploid chromosome number could slightly contribute to increase the generation of variability, as a consequence of the independent chromosome assortment (interchromosomal recombination). Therefore, we suggest that in both sexes, the main source of genetic variation would be interchromosomal recombination.

The degree of host-specificity amongst lice is high and many monophyletic groups of lice occur on monophyletic groups of hosts (Light et al. 2008). *P. h. capitis* and *P. h. humanus* occupy two environments, head and body, on the same host that are not alike, and are well-adapted to these environments (Schaefer 1978). In accordance with these, the achiasmatic nature of male meiosis brings about a diminution of intrachromosomal recombination, keeping together particular combinations of alleles, which are probably coadapted and function as supergenes (Ituarte and Papeschi 2004). The limited potential for genetic variability can be related to the high host specificity of these ectoparasites because a single species infests only one host (Tombesi and Papeschi 1993). At the same time, this type of meiosis could be an adaptive strategy of these species to the particular type of habitat and ecological niche they occupy.

The male of *P. h. humanus* transmits to their offspring only their maternal set of chromosomes because their paternal set is eliminated during development (McMeniman and Barker 2006). This is also called transmission ratio distortion because there is a statistically significant deviation in the expected offspring genotype inheritance in excess of the expected Mendelian proportion of 50%. The authors suggested that a possible explanation of its effect might be the presence of B chromosomes that eliminates paternally derived chromosomes. We observed a regular behavior of the studied chromosomes of both head and body lice. Thus, no B or supernumerary chromosomes were observed in all the studied insects.

The comparison of the transcriptional profiles of both lice showed that of the nine genes with differential expression, only one gene is missing (PHUM540560) that encodes a protein of unknown function in the head lice (Olds et al. 2012). More recently, Drali et al. (2013) found significant differences between head and body lice from a global collection in the sequence PHUM540560. Thus, it would be interesting to use this sequence as a chromosome marker for a better knowledge of chromosome structure and organization among human lice.

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