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Brief report
Chromosome numbers of two sucking louse species (Insecta, Phthiraptera, Anoplura)

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Sucking and chewing, or biting lice – Anoplura and Mallophaga (Phthiraptera) – are highly exceptional insects in respect to their chromosome systems. Representatives of these groups display holocentric chromosomes and achiasmatic male meiosis. In addition, spermatogenesis is of unique type with several mitotic divisions after the first meiotic division resulting in the formation of cysts with functional and non-functional "tertiary spermatids" (Tombesi and Papeschi 1993; Tombesi et al. 1999). The number of "tertiary spermatids" in a cyst, 64 or 128, is considered as a species specific character (Tombesi and Papeschi 1993). So far, only few cytogenetic studies on Phthiraptera have been carried out, and only 7 species of Anoplura and 6 species of Mallophaga have been karyotyped (Tombesi and Papeschi 1993). In this study, we represent the first data on karyotypes and spermiogenesis of two anopluran species, *Polyplax serrata* Burm. (Polyplacidae) and *Hoplopleura* sp. (Hoplopleuridae).

MATERIAL AND METHODS

Adult males and females of *Polyplax serrata* Burm. (Polyplacidae) were collected from *Apodemus* field mouse in Caucasian Reserve (Russia) in 2003. Specimens of *Hoplopleura* sp. (Hoplopleuridae) were collected from *Apodemus* in the vicinity of Novgorod (Russia) in 2000. All specimens were fixed in an alcohol/acetic acid mixture (3 parts of 96% ethanol: 1 part of glacial acetic acid) and kept in refrigerator at +4°C. Individual testicular follicles, oocytes and matured eggs were squashed on slides in a drop of 45% or 60% acetic acid. Before squashing, the chorion was carefully removed from mature eggs. After cover slips were removed by dry ice technique (Conger and Fairchild 1953), slides were air-dried and stained by Feulgen-Giemsa procedure (Grozeva and Nokkala 1996). In brief, slides were incubated in 1N HCl at room temperature for 20 min, hydrolysed in 1N HCl at 60°C for 7 min, stained with Schiff's reagent for 30 min, thoroughly rinsed in distilled water and Sorensen's phosphate buffer, pH 6.8. Finally, slides were stained with 5% Giemsa solution for 20–30 min. After staining, slides were rinsed briefly with distilled water, air dried, and mounted in Entellan.

RESULTS AND DISCUSSION

The species *P. serrata* and *Hoplopeura* sp. showed similar cytological characters in males and females. Testes consisted of two follicles and ovaries of five ovarioles. Our data on *P. serrata* (Polyplacidae) confirmed previous observations on this species (Emeljanov et al. 2001), whereas the data on *Hoplopleura* sp. are reported for the first time and represent the first information of this kind for the family Hoplopleuridae as a whole. Testes with two follicles are characteristic for all studied species of Anoplura and for a part of Mallophaga, whereas ovaries consisting of five ovarioles are characteristic for all phthirapterans (Blagoveshchenskii 1956; Emeljanov et al. 2001).

Within females of *P. serrata* and *Hoplopleura* sp. the mature eggs contained early embryos at different ages. Thus, ovoviviparity can be assumed as characteristic for species studied. The embryos could comprise up to 32 cells suggesting the presence of the 5th cleavage divisions.

In oocytes, metaphase I (MI) showed 8 bivalents (Fig. 1a and b). Bivalents gradually decreased in size and displayed each one chiasma. One could not identify the sex chromosome bivalent. Thus, chromosome formula of the female diploid karyotype was determined as $2n = 16$ (14+XX) for both species. Previously, this chromosome number was unknown in Phthiraptera.

In adult males of *P. serrata* and *Hoplopeura* sp. testes contained cysts with 64 "tertiary spermatids",

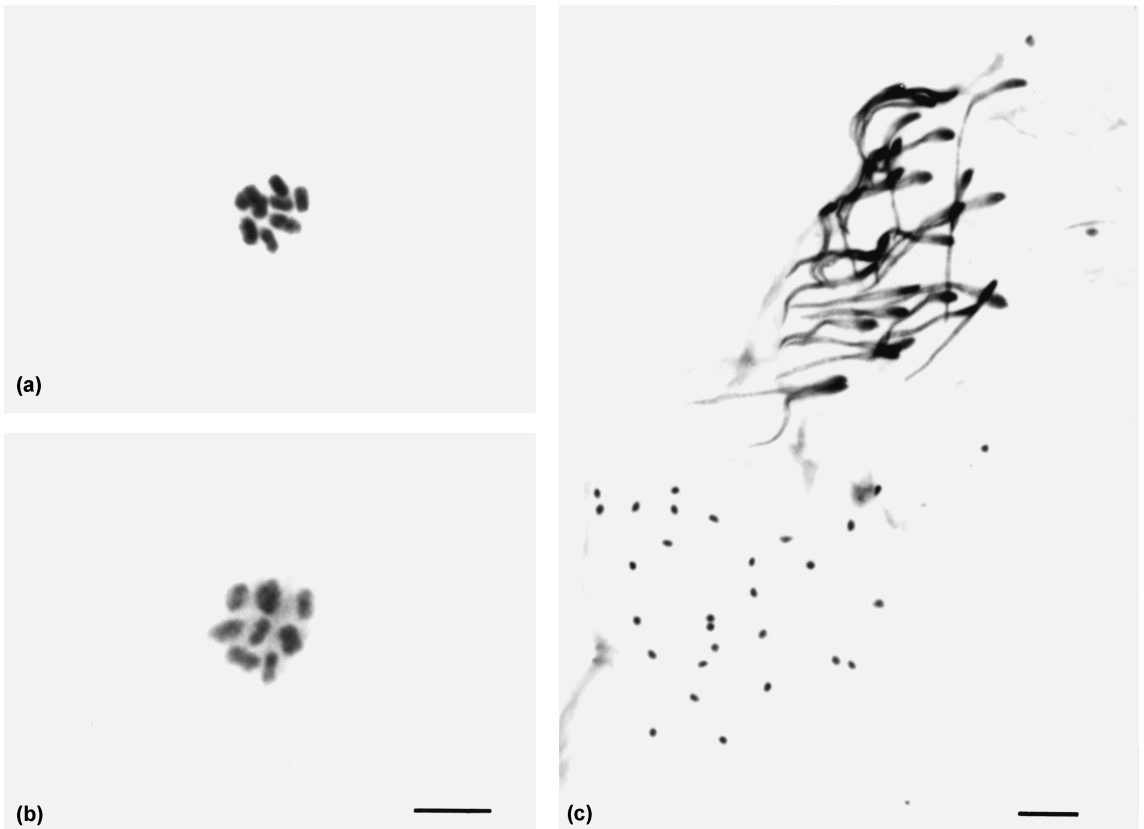

Fig. 1. Female meiotic chromosomes and spermatogenesis in two Anoplura species (**a**) oocyte M I in of *P. serrata*, (**b**) oocyte M I in of *Hoplopleura* sp., (**c**) spermatogenesis in *P. serrata*: a cyst with 32 functional spermatozoa and 32 non-functional pycnotic nuclei. Scale bar equals 5 μm (a–b) or 10 μm (c).

32 functional and 32 non-functional "pycnotic" nuclei (Fig. 1c), and cysts with mature sperms. This observation is consistent with previous findings (Bayreuther 1955; Tombesi and Papeschi 1993) that in male Phthiraptera, the first meiotic division is followed by four or five mitotic divisions resulting in cysts with 32 or 64 haploid spermatocytes, respectively. Subsequently, spermatocytes undergo one further "unequal mitotic division", which results in the appearance of 64 or 128 "tertiary spermatids", respectively. This unique process leads to the formation of 32 or 64 functional sperms and 32 or 64 additional "pycnotic" nuclei.

The data on Phthiraptera karyotypes have been summarized by Tombesi and Papeschi (1993). More recently only one paper dealing with karyotypes of *Bovicola limbata* and *B. caprae* (Mallophaga) has been published (Tombesi et al. 1999). With our new data, karyotypes of 15 species, including 9 species from 5 genera and 5 families of Anoplura and 6 species from 5 genera and 4 families of Mallophaga, are known. This comprises less than 0.5% of described phthirapteran species.

Phthiraptera seem to be characterized by quite large diversity of chromosome numbers. In 15 studied species as much as 7 different chromosome numbers varying from $2n=4$ to $2n=24$ have been found (Tombesi and Papeschi 1993; Tombesi et al. 1999; this paper). The modal chromosome number in Phthiraptera cannot be presently established because of the scarcity of data available. In all genera, except *Haematopinus*, *Pediculus* (Anoplura) and *Bovicola* (Mallophaga), only a single species has been karyotyped. In *Haematopinus* all three species studied show different chromosome numbers $2n=10$, 14, and 18 in *H. suis*, *H. consobrinus* and *H. asini*, respectively. In *Pediculus* both *P. capitis* and *P. corporis* have $2n=12$, while *P. vestimenti* have $2n=10$. The both *Bovicola* species studied, *B. limbata* and *B. caprae*, have $2n=14$.

Phthiraptera represent a closely related group to Psocoptera (Kristensen 1981). It is worth noting, that the karyotype with $2n=16$ found in *P. serrata* and *Hoplopeura* sp. (Anoplura) occurs in the psocopteran family Liposcelidae (Jostes 1975), which was recently suggested as a probable

sister group of Phthiraptera (Yoshizawa and Johnson 2003).

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REFERENCES

Bayreuther, K. 1955. Holokinetische Chromosomen bei *Haematopinus suis* (Anoplura, Haematopinidae). – Chromosoma 7: 260–270.

Blagoveshchenskii, D. I. 1956. Structure and taxonomic significance of the reproductive system in birdlice (Mallophaga). – Parasitol. Sb. Moscow 16: 5–88.

Conger, A. D. and Fairchild, L. M. 1953. A quick-freeze method for making smear slides permanent. – Stain. Technol. 28: 289–293.

Emeljanov, A. F., Golub, N. V. and Kuznetsova, V. G. 2001. Evolutionary transformation of testes and ovaries in booklice, birdlice, and sucking lice (Psocoptera, Phthiraptera: Mallophaga, Anoplura). – Entomol. Rev. 80: 767–785.

Grozeva, S. and Nokkala, S. 1996. Chromosomes and their meiotic behaviour in two families of the primitive infraorder Dipsocoromorpha (Heteroptera). – Hereditas 125: 31–36.

Jostes, R. F. 1975. A method for determining the chromosome number of parthenogenetic psocids (Insecta, Psocoptera). – Cytologia 40: 553–555.

Kristensen, N. P. 1981. Phylogeny of insects orders. – Annu. Rev. Entomol. 26: 135–157.

Tombesi, M. L. and Papeschi, A. G. 1993. Meiosis in *Haematopinus suis* and *Menacanthus stramineus* (Phthiraptera, Insecta). – Hereditas 119: 31–38.

Tombesi, M. L., Papeschi, A. G. and Mola, A. M. 1999. Spermatogenesis in *Bovicola limbata* Gervais, 1844 and *B. caprae* Gurlt, 1843 (Phthiraptera, Ischnocera). – Cytologia 64: 25–27.

Yoshizawa, K. and Johnson, K. P. 2003. Phylogenetic position of Phthiraptera (Insecta: Paraneoptera) and elevated rate of evolution in mitochondrial 12S and 16S rDNA. – Mol. Phylogenet. Evol. 29: 102–114.