A brief karyological review of the Gracillariidae (Lepidoptera)

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Abstract. The present paper reports the data on the haploid chromosome numbers of 14 species from 4 genera of the Gracillariidae, collected in Central Asia and Lithuania. All the descriptions of the karyotypes are presented here for the first time.

Samenvatting. Een korte studie van de karyologie bij de Gracillariidae (Lepidoptera) Het artikel bevat de haploïde chromosoomaantallen van 14 soorten uit 4 genera van de Gracillariidae, die werden verzameld in Centraal-Azië en Lithouwen. Alle gegevens van deze karyotypes worden hier voor het eerst gepubliceerd.

Résumé. Une étude karyologique des Gracillariidae (Lepidoptera)
Les données sur le nombre haploïde de chromosomes chez 14 espèces de 4 genres appartenant à la famille des Gracillariidae, collectionnées en Asie Centrale et en Lituanie, sont rapportées. Toutes les descriptions des karyotypes sont présentées pour la première fois.

Key words : Gracillariidae - *Gracillaria* - *Caloptilia* - *Phyllonorycter* - *Cameraria* - karyotypes - chromosome numbers.

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Introduction

Early studies of the karyotypes of the Gracillariidae are particularly scanty. So far the germ-line chromosome number has been known in *Caloptilia elongella* (LINNAEUS, 1761). Its haploid chromosome number n=29 was recorded by BELIAEFF (1930). The poor karyological investigation of mining Lepidoptera hampers the analysis of the karyotypic evolution in the primitive Microlepidoptera. The present study reports some data on the haploid chromosome numbers of 14 species from 4 genera of the Gracillariidae. The paper envolves two phylogenetically related genera (*Gracillaria* HAWORTH, 1828 and *Caloptilia* HÜBNER, [1828]) in subfamily Gracillariinae and two closely related genera (*Phyllonorycter* HÜBNER, 1822 and *Cameraria* CHAPMAN, 1902) in subfamily Lithocolletinae. All the descriptions of the karyotypes are presented for the first time.

Material and methods

The material was collected in Tadzhikistan, Turkmeniya and Lithuania. Larvae were reared till suitable stage. Only males of the Gracillariidae were used for the karyological study. Testes of larvae or pupae were fixed in ethanolacetic solution (3:1) and were kept from one week till two months. Further testes were stained in 2% aceto-orcein medium. The squashed temporary preparations were made in a drop of 45% acetic acid. The chromosomes were examined in the metaphase I (MI) or metaphase II (MII) of the meiosis of spermatogenesis.

The brief karyological characteristics of the Gracillariidae

The chromosomes of the Gracillariidae, as usual for the other Lepidoptera, are holocentric, possessing no definite centromeric region. The bivalent chromosomes of the Gracillariidae are rather small. Usually they are dot-shaped, or show characteristic 8-shaped form. The frequency of the haploid chromosome numbers of the Gracillariidae moths is commonly stable, not variable. The number n=30 stands out in the investigated species and is the most characteristic for this family. Also this haploid chromosome number is mentioned for the other Microlepidoptera (ROBINSON 1971). So the modal chromosome number of the Gracillariidae could be n=30.

Usually the bivalents in the complements gradually decrease in size. But the complements of the several species are characterized by the inclusion of one large bivalent or several elements, which are considerably larger than the rest. The appearance of the larger bivalent and the reduction of the haploid chromosome number (*Phyllonorycter populifoliella* (TREITSCHKE, 1833)) might be involved in the process of fusion.

Almost each investigated species possesses a normal complement of chromosomes, with the exception of *Phyllonorycter dubitella* (HERRICH-SCHÄFFER, 1855), which shows a mosaic karyotype. One additional or B chromosome, which has a number of properties, which distinguish it from the normal chromosomes might be present in this species. An additional chromosome is smaller than usual and stains more faintly, behaves irregularly at meiosis (does not form a bivalent).

Genus *Gracillaria* HAWORTH, 1828

This genus, distributed throughout Holarctic, includes about 7 species. Seven species are known in the Palaearctic fauna.

Gracillaria syringella (FABRICIUS, 1794) (fig. 1) :
Vilnius, Lithuania, 10.VII.1992. Larvae were collected on *Syringa vulgaris*. The first primary spermatocyte divisions were observed in the late pupa. The metaphase plate is considerbaly smaller than in the other miners. The chromosomes are very small, dot-shaped. The complement is characterized by the inclusion of several larger bivalents. The haploid chromosome number n=30 was determined in four metaphases of two males.

Genus *Caloptilia* HÜBNER, [1825]

The genus has worldwide distribution. About 300 species are known. More than 100 species are known in the Palaearctic fauna.

Caloptilia rufipennella (HÜBNER, 1796) (fig. 2) :
Vilnius, Lithuania, 09.VII.1991. Larvae were collected on *Acer platanoides*. The counts of chromosome numbers were made in the metaphase plates of testes of pupae. The haploid chromosome number n=30 was determined after the examination of 14 metaphases in three males. The bivalents are elongated or 8-shaped, gradually decrease in size.

Genus *Phyllonorycter* HÜBNER, 1822

The genus has a worldwide distribution and includes about 500 species. More than 200 species are known in the Palaearctic fauna.

Phyllonorycter asiatica (GERASIMOV, 1931) (figs 3-5) :
Ashkhabad, Turkmeniya, 01.XII.1991. The pupae were collected on *Salix*

spp. The primary spermatocyte divisions were found in pupa. The haploid chromosome number n=30 was determined after examination of 30 metaphases in 5 males. Most of the bivalents are 8-shaped, since one of them is more faintly stained (figs 3, 4). Besides, one chiasma is shown (fig. 3). All the bivalents gradually decrease in size till very small ones (figs 3, 4). The speed of separation of the bivalents is very different (fig. 5).

Phyllonorycter sorbi (FREY, 1855) (fig. 6) :
Ignalina, Lithuania, 22.X.1991. Larvae were collected on *Sorbus aucuparia*. The primary spermatocyte divisions were noticed in pupae. The determined haploid chromosome number is n=30, based on counts of 19 metaphases in 6 males. The bivalents are mostly dot-shaped, gradually decrease in size. Two of them are considerably smaller.

Phyllonorycter lautella (ZELLER, 1846) (fig. 7) :
Klaipeda, Lithuania, 01.VII.1992. Larvae were collected on *Quercus robur*. The primary spermatocyte divisions were found in the last instar larvae. The haploid chromosome number n=30 was determined in the counts of 3 metaphases in two males. The bivalents gradually decrease in size.

Phyllonorycter blancardella (FABRICIUS, 1781) (figs 8-10) :
Ignalina, Lithuania, 28.IX.1991. Vilnius, Lithuania, 13.X.1991. Larvae were collected on *Malus domestica*. The metaphase plates were examined in pupa. The haploid chromosome number was determined in counts, based on 29 metaphases in 12 males. The bivalent chromosomes are dot-shaped or 8-shaped as usual. Several bivalents, which are larger than the rest and 5-6 little bivalents exclude in the complement of this species (figs 8, 9). The other bivalent chromosomes are more or less equal in size.

Phyllonorycter ulmifoliella (HÜBNER, [1817]) (figs 11, 12) :
Ignalina, Lithuania, 23.IX.1991. Larvae were collected on *Betula pendula*. The primary spermatocyte divisions were found in the last instar larvae. The haploid chromosome number n=30 was determined in 28 metaphases of 12 males. All the bivalents are elongated or dumb-bell shaped and form a decreasable row in size. The largest bivalent separates the first in the set (fig. 12).

Phyllonorycter cerasicolella (HERRICH-SCHÄFFER, 1855) (fig. 13) :
Ashkhabad, Turkmeniya, 01.XII.1991. Larvae were collected on *Prunus domestica*. The first primary spermatocyte divisions were noticed in the midinstar larvae, which were fixed and examined a month after the collection, on 03.II.1992. The bivalents are elongated, gradually decrease in size, though four pairs show later contraction. The haploid chromosome number n=30 was determined, based on counts of 13 metaphases in 12 males.

Phyllonorycter rajella (LINNAEUS, 1758) (figs 14, 15) :
Ignalina, Lithuania, 28.IX.1991. Vilnius, Lithuania, 13.X.1991. Larvae were collected on *Alnus incana*. The primary spermatocyte divisions were found in the last instar larvae and in early pupae. The haploid chromosome number determined as n=30, based on counts of 29 metaphases in 14 males. The

bivalents are dot-shaped or oval. One of them is considerably larger, 4-5 bivalents are smaller in comparison with the rest, which are almost equal in size. Sometimes the bivalents form one-three chains in the complements of *Ph. rajella*.

Phyllonorycter sylvella (HAWORTH, 1828) (fig. 16) :
Vilnius, Lithuania, 17.X.1991. Larvae were collected on *Acer platanoides*. The primary spermatocyte divisions were examined in pupae. The haploid chromosome number n=30 was counted in 34 metaphases of 11 males. The bivalents are oval or 8-shaped and gradually decrease in size.

Phyllonorycter dubitella (HERRICH-SCHÄFFER, 1855) (figs 17-18) :
Vilnius, Lithuania, 07.VII.1992. Larvae were collected on *Salix caprea*. The divisions of primary spermatocytes were detected in the last instar larvae and in pupae. The bivalents are mostly 8-shaped or oval. The haploid chromosome number n=30 was determined in counts of 9 metaphases in 3 males. The complement of the chromosomes is characterized by the inclusion of one bivalent, which is considerably larger than the rest. The other bivalents gradually decrease in size. The speed of separation in the bivalents of the set is different; the largest bivalent separates the last (fig. 17). The karyotype of *Ph. dubitella* tends to be mosaic. Some of the complements of this species possess an accessory, correspondingly smaller element. Besides, several chromosomes might lay as chromosome chains of two bivalents or one trivalent. The characteristic features of the karyotype of *Ph. dubitella* could be an appearance of one large bivalent, an occurrance of an accessory element, owing to the fission and fusion of the chromosomes.

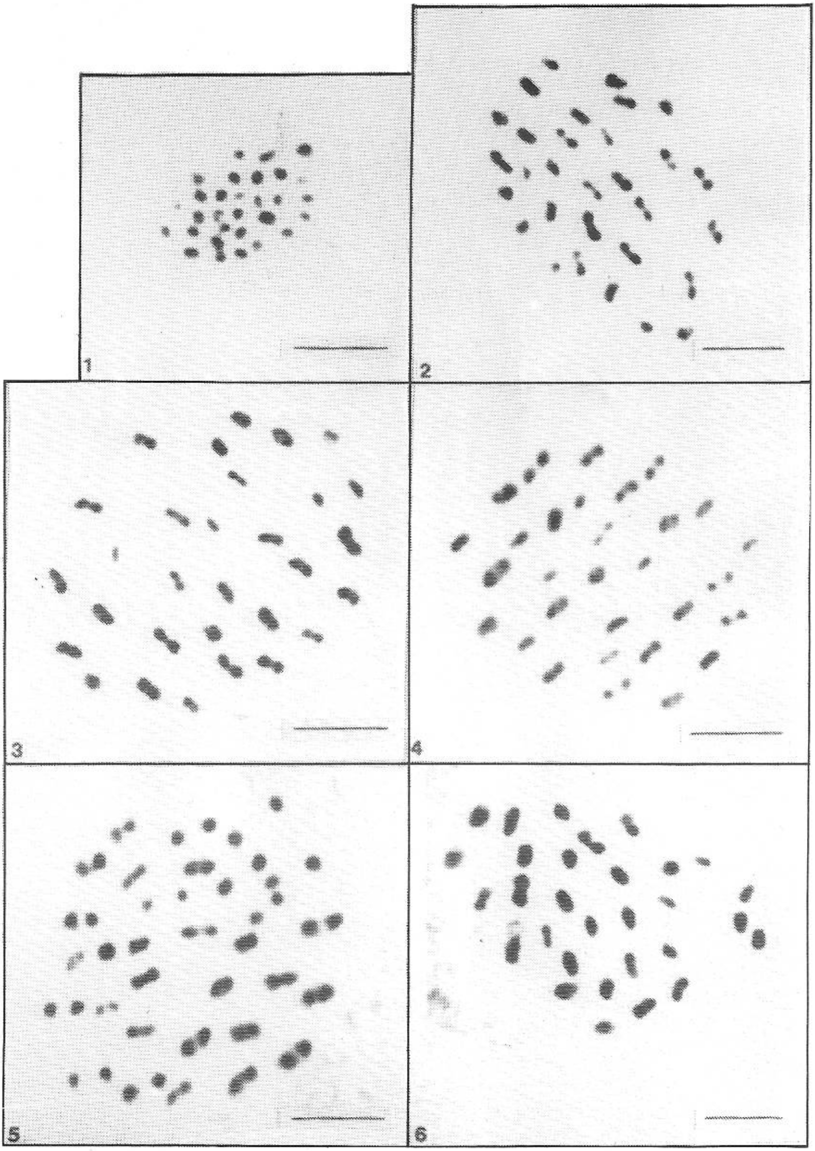
Phyllonorycter populifoliella (TREITSCHKE, 1833) (figs 19-21) :
Vilnius, Lithuania, 13.VII.1991. Larvae were collected on *Populus balsamifera*. The divisions of the primary spermatocytes were first observed in the prepupae. The haploid chromosome number n=29 was determined in the counts of 40 metaphases in 12 males. The complement is characterized by the presence of the oval-shaped large bivalent. The rest ones are 8-shaped and gradually decrease in size.

Genus *Cameraria* CHAPMAN, 1902

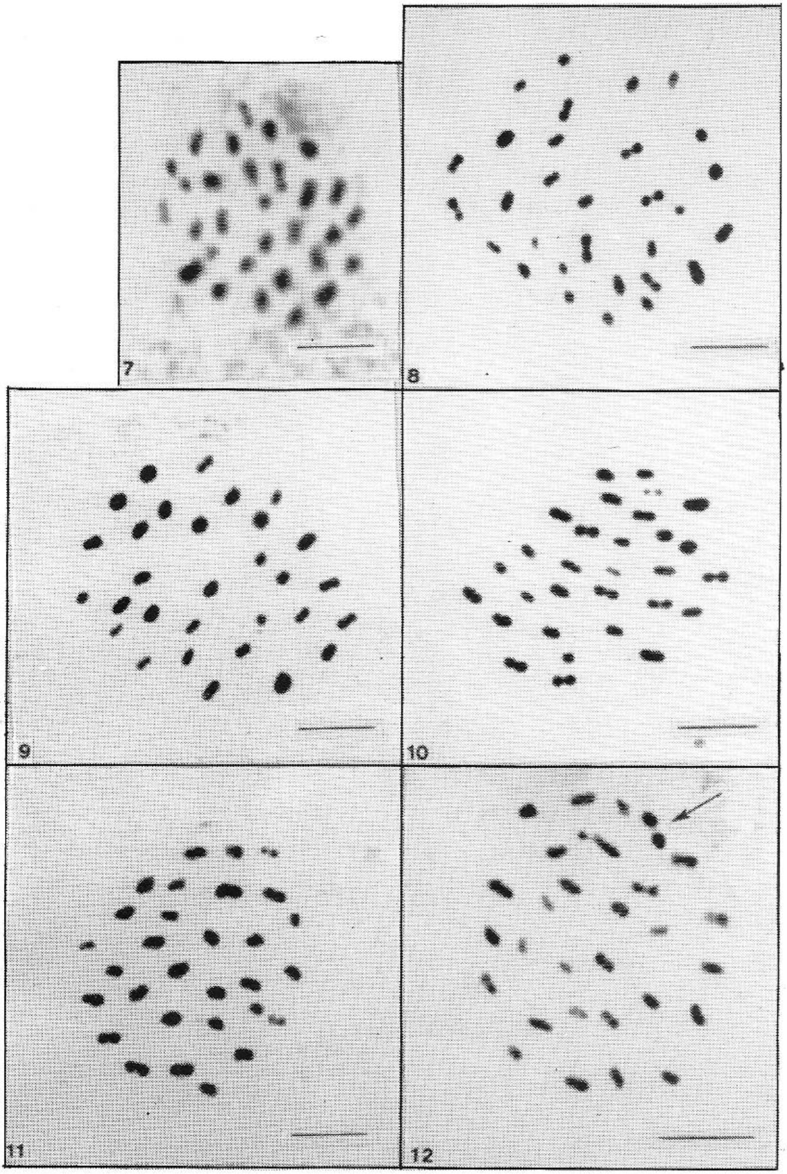
The genus has Holarctic distribution. Six species are known in the Palaearctic fauna.

Cameraria saliciphaga (KUZNETSOV, **1975) (fig. 22)** :
Kurgan Tjube, Tadzhikistan, 07.VII.1990. Larvae were collected on *Salix* spp. The primary spermatocyte divisions were determined in the late instar larvae. The haploid chromosome number n=30 was counted in 15 metaphases of 10 males. The bivalents are dot-shaped or elongated as usual. All the bivalents gradually decrease in size till very tiny ones. The metaphase plates of *C. saliciphaga* are small, much more smaller than in *C. obliquifascia*.

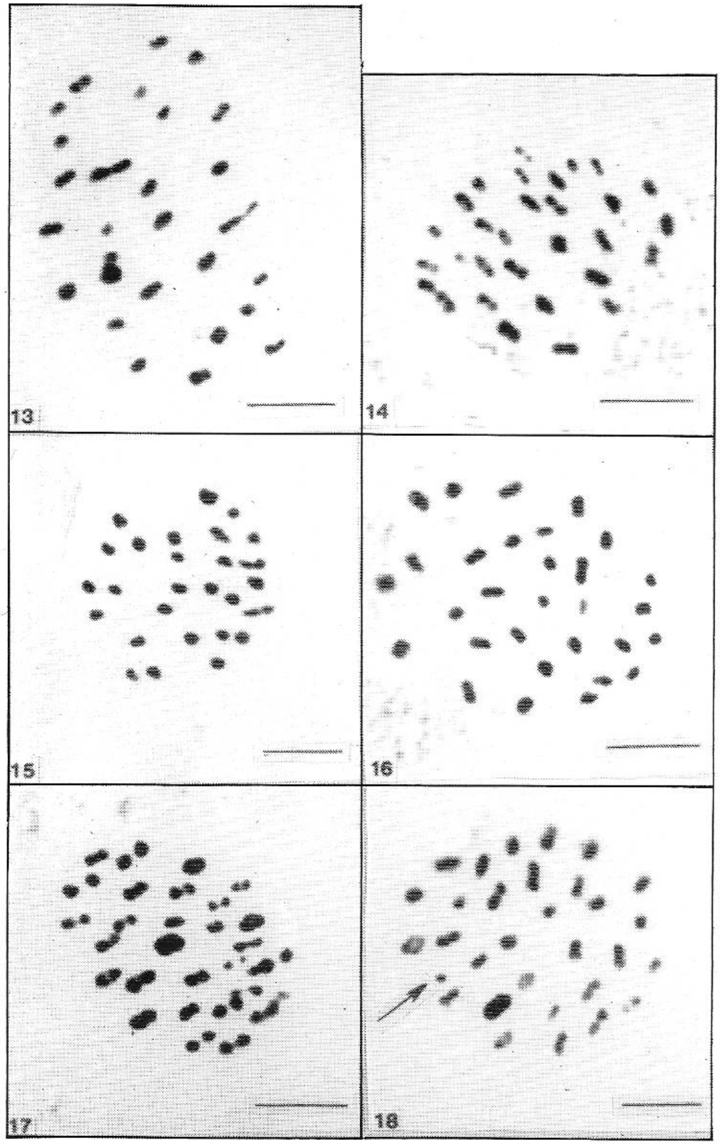
Cameraria obliquifascia (FILIPJEV, **1926) (fig. 23)** :
Askhabad, Turkmeniya, 01.XII.1991. Larvae were collected on *Salix* spp. The beginning of the primary spermatocyte divisions was recorded in larvae,



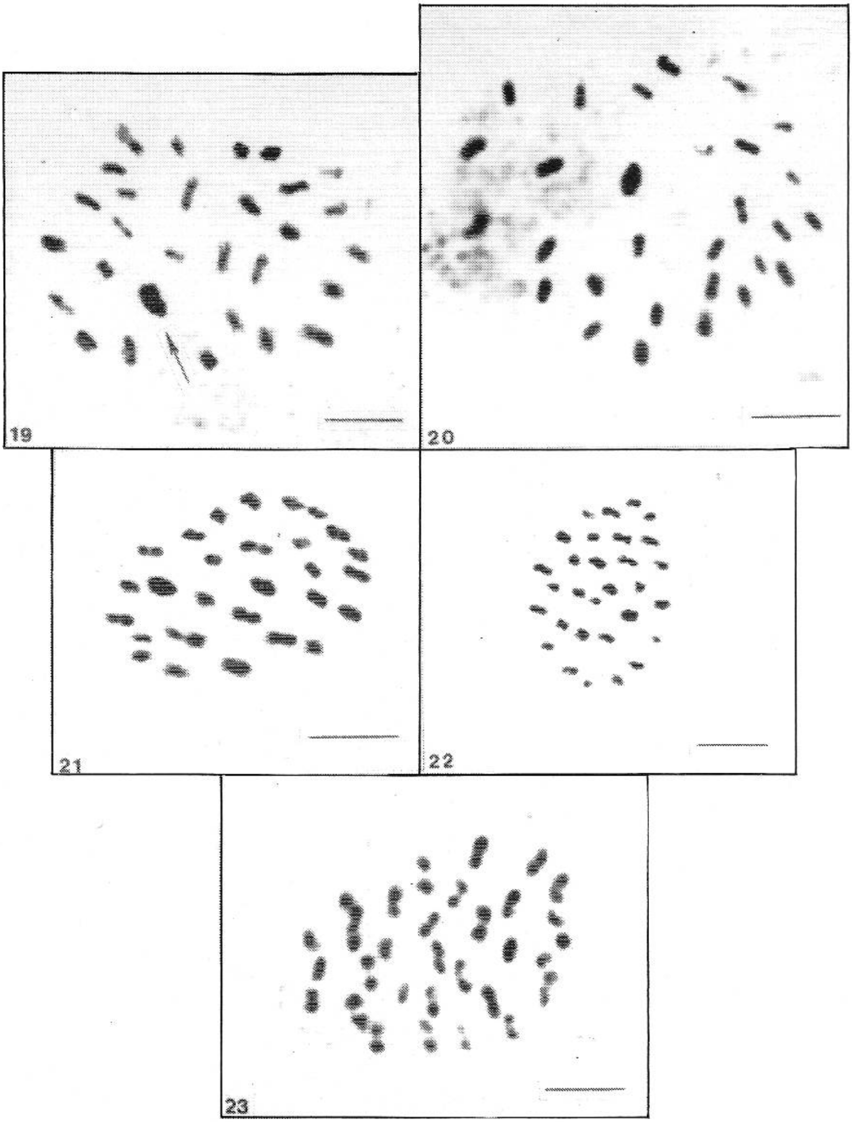
Figs 1-6 : Primary spermatocyte chromosomes of *Gracillaria, Caloptilia* and *Phyllonorycter* : 1. *Gracillaria syringella* (FABRICIUS, 1794), n=30; 2. *Caloptilia rufipennella* (HÜBNER, 1796), n=30; 3-5. *Phyllonorycter asiatica* (GERASIMOV, 1931), n=30; 5. Different separation of the bivalents in the complement of *Ph. asiatica*. 6. *Phyllonorycter sorbi* (FREY, 1855), n=30; orcein-stained, scale line ca. 5 µm.



Figs 7-12 : Primary spermatocyte chromosomes of *Phyllonorycter* : 7. *Phyllonorycter lautella* (ZELLER, 1846), n=30; 8-10. *Phyllonorycter blancardella* (FABRICIUS, 1781), n=30; 11-12. *Phyllonorycter ulmifoliella* (HÜBNER, [1817]), n=30. 12. An arrow shows the largest bivalent in the complement of *Ph. ulmifoliella*; orcein-stained, scale line ca. 5 µm.



Figs 13-18 : Primary spermatocyte chromosomes of *Phyllonorycter* : 13. *Phyllonorycter cerasicolella* (HERRICH-SCHÄFFER, 1855), n=30; 14-15. *Phyllonorycter rajella* (LINNAEUS, 1758), n=30; 16. *Phyllonorycter sylvella* (HAWORTH, 1828), n=30; 17-18. *Phyllonorycter dubitella* (HERRICH-SCHÄFFER, 1855), n=30; 18. An arrow shows the accessory element in the complement of *Ph. dubitella*, n=30+m; orcein-stained, scale line ca. 5 µm.



Figs 19-23 : Primary spermatocyte chromosomes of *Phyllonorycter* and *Cameraria* : 19-21. *Phyllonorycter populifoliella* (TREITSCHKE, 1833), n=29; 19. An arrow shows the large bivalent in the complement of *Ph. populifoliella*; 22. *Cameraria saliciphaga* (KUZNETSOV, 1975), n=30; 23. *Cameraria obliquifascia* (FILIPJEV, 1926), n=30; orcein-stained, scale line ca. 5 µm.

on 07.IV.1992. The haploid chromosome number n=30 was determined, based on counts of 8 metaphases in two males. The bivalents are large in comparison with the other miners. Most of them are 8-shaped. All the bivalents gradually decrease in size, the larger ones separate more rapidly.

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