

Karyotype characteristics and polymorphism peculiarities of *Chironomus bernensis* Wülker & Klötzli, 1973 (Diptera, Chironomidae) from the Central Caucasus and Ciscaucasia

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Abstract

Data about the karyotype characteristics, features of chromosomal polymorphism and larval morphology of populations of *Chironomus bernensis* Wülker & Klötzli, 1973 (Diptera, Chironomidae) from the Central Caucasus (the northern macroslope) and Ciscaucasia are presented. The characteristics of the pericentromeric regions of the long chromosomes of this species from Caucasian populations were very similar to the ones from some European populations (from Poland and Italy), but differed from Swiss and Siberian populations. In the North Caucasian populations 10 banding sequences were found: two in arms A, C, and E, and one in arms B, D, F, and G. Nine of them were already known for this species, and one, berC2, is described for the first time. Cytogenetic distances between all the studied populations of *Ch. bernensis* show that close geographical location of all studied populations from the Central Caucasus and Ciscaucasia is reflected in their similar cytogenetic structure, but on the other hand, that they are more closely related to populations from Europe than to populations from Western Siberia. At the same time, all studied larvae from Caucasian populations have a four-bladed premandible, instead of a two-bladed one, as in the description of *Ch. bernensis* from Switzerland (Wülker and Klötzli 1973, Polukonova 2005c). These peculiarities may indicate the relative isolation of the Caucasus from the viewpoint of microevolution. Further research on karyological and morphological characteristics of *Chironomus bernensis* from geographically distant regions is necessary as there is a possibility that the presently known species is actually polytypic and consists of several sibling species.

Keywords

Diptera, Chironomidae, *Chironomus bernensis*, polytene chromosomes, chromosome polymorphism, pericentromeric region, cytogenetic distances, larva premandible, Central Caucasus (northern macroslope), Ciscaucasia

Introduction

Chironomus bernensis was first described by Wülker and Klötzli in 1973 from Switzerland (Wülker and Klötzli 1973). The species belong to the “lacunarius” cytocomplex ($2n=8$, chromosome arm combinations AD, BC, EF, G).

The karyotype of *Ch. bernensis* was studied early-on from Switzerland (Wülker and Klötzli 1973), Bulgaria, Poland, Northern Italy (Michailova 1989, Michailova et al. 2002, Petrova and Michailova 2002, Michailova et al. 2009) and Spain (Real et al. 2000). In Russia this species was known only from Western Siberia and the chromosomal polymorphism of those populations was described by Istomina and Kiknadze (Istomina and Kiknadze 2004, Kiknadze et al. 2007).

The aim of this work is to present the description of karyotype, chromosomal polymorphism and larval morphology of *Ch. bernensis* from the Central Caucasus (the northern macroslope) and Ciscaucasia – Republic of Kabardino-Balkaria (RKB), Republic of North Ossetia-Alania (RNO-Alania), Karachai-Cherkess Republic (KCR) and Stavropol Krai. It was also important to compare characteristics of chromosomal polymorphism of *Ch. bernensis* from Caucasus, Western Europe and Western Siberia.

Methods

Fourth instar larvae were used in the karyological study. The larvae were collected from 12 sites of the Central Caucasus and Ciscaucasia: seven sites from Republic of Kabardino-Balkaria (RKB), one site from Republic of North Ossetia-Alania (RNO-Alania), one site from Karachai-Cherkess Republic (KCR), and four sites from Stavropol Krai (Table 1). In the aspect of the vertical zonation the site in KCR belongs to the Kuban variant, all sites in Stavropol Krai belong to the steppe zone and all sites in RKB and RNO-Alania belong to the Terek variant (typification of the zone variants are given according to Sokolov and Tembotov 1989).

In total 85 specimens of *Ch. bernensis* were studied.

For karyotype analysis larvae were fixed in ethanol-glacial acetic acid (3:1). Slides of the chromosomes were prepared with ethanol-orcein technique (Dyomin and Ilyinskaya 1988, Dyomin and Shobanov 1990).

The identification of chromosome banding sequences for arms A, E and F was performed with use of photomaps of Wülker and Klötzli (1973) in the system of Keyl (Keyl 1962) and chromosome mapping for arms C and D was performed according to Istomina and Kiknadze (2004) in the system of Dévai et al. (Dévai et al. 1989). Micro-

Table 1. Collection sites and number of specimens of *Chironomus bernensis* of Central Caucasus.

Localities	Collection sites	Collection date	Number of specimens
RKB	43°27.05'N; 43°35.42'E, mouth of Nartia River, near Khasania village, altitude ca 440 m a.s.l.	21.12.07	3
	43°37.44'N; 43°55.09'E, main riverbed of Urvan River, near Koldrasynkyi hamlet, altitude ca 230 m a.s.l.	29.07.08	1
	43°22.59'N; 43°42.77'E, floodplain pool in riverbed of Kheu River, near Aushiger village, altitude ca 560 m a.s.l.	23.03.08	1
	43°29.16'N; 43°38.57'E, main riverbed of Nalchik River, Nalchik city, altitude ca 340 m a.s.l.	09.03.08	5
	43°45.02'N; 44°00.29'E, Prokhladnyi city, Vinzavod township, canal, altitude ca 200 m a.s.l.	18.02.09	1
	43°41.76'N; 44°00.39'E, former riverbed in mouth of Cherek River, near Oktyabrskiy village, altitude ca 210 m a.s.l.	21.03.10	9
	43°12.89'N; 43°39.37'E, 500 m over Zhemtala village, long-term waterbody, altitude ca 940 m a.s.l.	18.07.12	39
Stavropol Krai	43°58.71'N; 43°21.12'E, reservoir at Etoko River, in Verkhnetambukanskyi village, altitude ca 440 m a.s.l.	02.04.10	1
	44°42.72'N; 41°49.46'E, floodplain pool of Kuban River, near Kochubeevskaya village, altitude ca 280 m a.s.l.	14.10.10	2
	44°10.44'N; 42°40.81'E, floodplain pool of Kuma River, near Suvorovskiy village, altitude ca 450 m a.s.l.	14.10.10	4
	44°59.88'N; 41°45.33'E, Sengeleevskoe reservoir, near Sengeleevskaya village, altitude ca 230 m a.s.l.	15.10.10	1
RNO-Alania	43°19.85'N; 44°11.19'E, bed of lowered pond near Zmeiskaya village, altitude ca 310 m a.s.l.	05.05.10	1
KCR	44°21.82'N; 41°55.96'E, backwater in main riverbed of Malyi Zelenchuk River, near Adyl-Khalk village, altitude ca 420 m a.s.l.	14.10.10	17

scope Carl Zeiss Axio Imager.A2 was used to study chromosome slides. Software packages PAST 2.17 and STATISTICA 10 were used for statistical analysis (cluster analysis).

The following parameters were used for comparison of characteristics of chromosomal polymorphism: the number of zygotic combinations, percentage of heterozygous larvae, number of heterozygous inversions per specimen, number of inversions per arm, number of banding sequences in a population.

Cytogenetic distances between populations were calculated according to Nei (Nei 1972).

Results

The larvae of the genus *Chironomus* Meigen, 1803 in all studied sites of the Central Caucasus and Ciscaucasia were attributed to *Ch. bernensis* by chromosomal and morphological characteristics. Morphological characteristics are presented on Fig. 1a–g. In

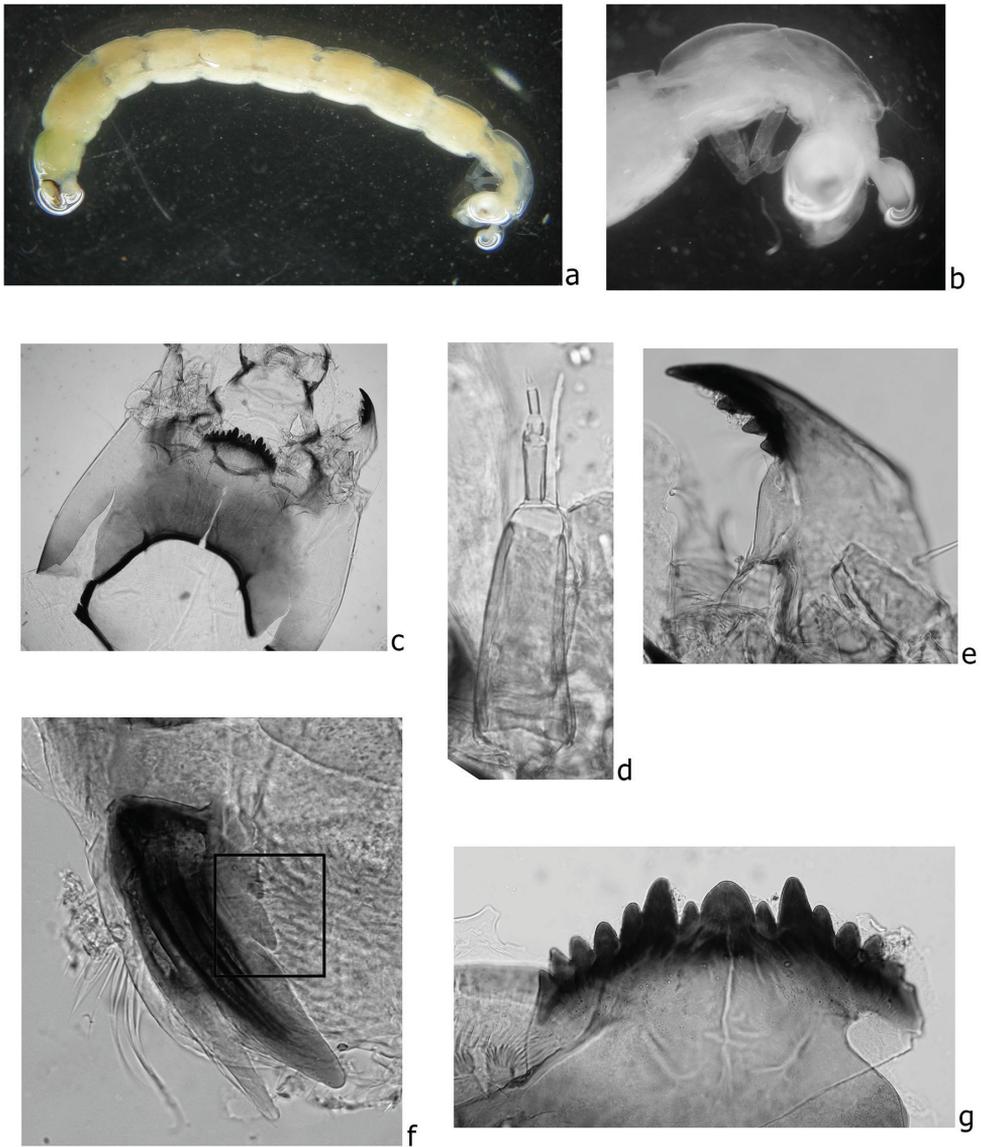


Figure 1. The larva of *Ch. bernensis* from the Central Caucasus and Ciscaucasia, **a** total view **b** ventral tubuli at segment VIII **c** head ventrally **d** antenna **e** mandible **f** premandible with additional teeth marked in the square **g** mentum.

general, the larval characters of *Ch. bernensis* from Caucasian sites are similar to those described previously for this species by Wülker and Klötzli (1973), however, some noticeable distinctions were found. Thus, it was stated by Wülker and Klötzli (1973) that larva of *Ch. bernensis* was not different from that of *Ch. commutatus*. Indeed, both species have the same type of larva (“bathophilus”), degree of gular sclerite pigmentation and structure of mentum and antenna. However, the fourth tooth of mandible

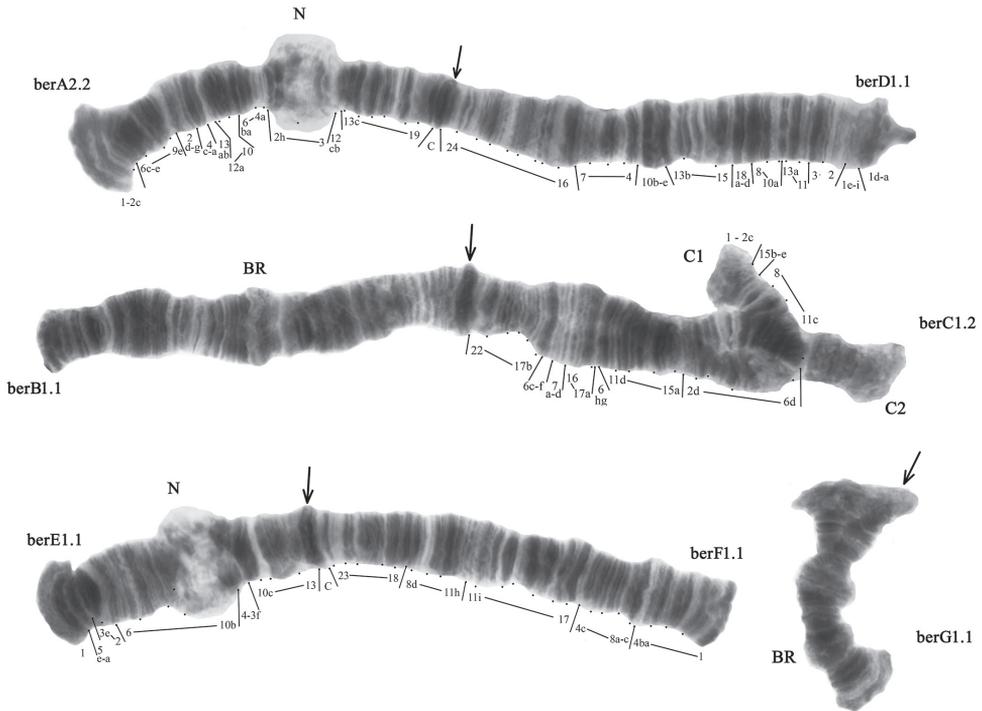


Figure 2. Karyotype of *Ch. bernensis* Northern Caucasus. berA2.2, berD1.1 etc. – zygotic combinations of banding sequences; BR – Balbiani rings, N – nucleoli. Arrows indicate centromeric regions.

of *Ch. bernensis* from Caucasian populations was dark brown or dark (Fig. 1e), while it is pale brown in *Ch. commutatus* according to Shobanov (2000). It is possible that Wülker and Klötzli (1973) did not notice this distinction. Another morphological peculiarity that was revealed was the presence of four-bladed premandibles in all studied larvae (Fig. 1f) instead of the two-bladed ones of *Ch. commutatus* (Laville 1971, Polukonova 2005c). The exterior tooth of the premandible in *Ch. bernensis* larvae of the North Caucasian populations was 2–2.5 times narrower than the inner one, longer and awl-shaped at the edge, the inner tooth was split into two small additional teeth near its basis (Fig. 1f).

Karyotype of *Ch. bernensis* from the Central Caucasus and Ciscaucasia

The diploid number of chromosomes in *Ch. bernensis* karyotype is $2n=8$, chromosome arm combination is AD, BC, EF, G – “lacunarius” cytocomplex (Fig. 2). Chromosomes AD and BC are metacentric, EF is submetacentric and G is telocentric. Two well developed nucleoli (N) are located on arms A and E. There are two Balbiani rings (BR) in the karyotype: one is situated in arm B and the other – in arm G, but in populations that we have studied the activity of both BR was greatly reduced (Fig. 2).

The centromeric bands of long polytene chromosomes of *Ch. bernensis* from the studied populations are large and belong to n-type (according to the classification by Shobanov (2002)). One of the peculiarities of the karyotype of *Ch. bernensis*, as indicate before by Istomina and Kiknadze (2004), is comparatively large telomeres of all chromosomes that often results in a presence of ectopic pairing between different chromosomes. We also observed such ectopic pairing with very low frequency and without any clear pattern between arms B and D in some specimens from different collection sites of Caucasus.

Banding sequences and chromosomal polymorphism of *Ch. bernensis* from the Central Caucasus and Ciscaucasia

Up until now, 16 banding sequences have been described in the banding sequences pool of *Ch. bernensis* (Table 2). In populations studied in this paper only 9 of those banding sequences were present, and one banding sequence has been found for the first time, so in total 10 banding sequences were found in Caucasian populations (Table 3).

Arm A. Two banding sequences – berA1 and berA2 – were found in both homozygous and heterozygous state (Fig. 3, Table 2–4). Banding sequence berA2 in homozygote (berA2.2) was dominant in all populations studied (Table 3, 4).

Arm B was monomorphic. Banding sequence berB1 remain unmapped due to the complex rearrangements that differ the banding pattern in the arm B of *Ch. bernensis* from the standard one of *Ch. piger*.

Table 2. Catalog of banding sequences in the banding sequences pool of *Ch. bernensis*.

Arm	Sequence	Order of bands	Authors of mapping
A	berA1	1-2c 10a-f 11-13ba 4a-c 2g-d 9e-6e-a-4d 2h-3i 12cb 13-19 C	Wülker and Klötzli 1973
	berA2	1-2c 6c-e-9e 2d-g 4a-c 13ab-11 10f-a 6ba-4d 2h-3i 12cb 13-19 C	-/-
B	berB1	Not mapped	-/-
C	berC1	1-2c 15b-e 8-11c 6b-2d 15a-11d 6gh 17a-16 7d-a 6f-c 17b-22 C	Istomina and Kiknadze 2004
	berC2	1-2c 4hi-6b 11c-8 15e-b 4g-a-2d 15a-11d 6gh 17a-16 7d-a 6f-c 17b-22 C	Original data
D	berD1	1a-d 1i-e 2-3 11-13a 10a-8 18d-a 15-13b 10b-e 4-7 16-17 18e-24 C	Istomina and Kiknadze 2004
E	berE1	1a-i 5e-a 3e-2 6-10b 4-3f 10c-13 C	Wülker and Klötzli 1973
	berE2	1a-i 5e-a 3e-2 6-10b 12-11 10g-c 3f-4h 13 C	Petrova and Michailova 2002
	berE3	1a-i 6ba 2-3a-e 5 6c-h-10b 4h-3f 10c-13 C	-/-
	berE4	1a-i 5e-a 3e-2 7d-6 7e10b 4-3f 10c-13 C	Istomina and Kiknadze 2004
F	berF1	1-4b 8c-4dc 17-12 11i-a-9f-c 8ed 18-23 C	Wülker and Klötzli 1973
	berF2	1-4b 8c-5d 11i-17 4c-5c 11h-10 9f-c 8ed 18-23 C	-/-
	berF3	1-4b 8c-4dc 11i-17 11h-8ed 18-23 C	Petrova and Michailova 2002
	berF4	1-4b 8c-5d 11i-15e 5a-4c 17d-15f 5bc 11h-10 9f-c 8ed 18-23 C	Istomina and Kiknadze 2004
G	berG1	1 2 3 4 7ba 6 5 7c-e	Petrova and Michailova 2002
	berG2	Not mapped	Istomina and Kiknadze 2004
	berG3	Not mapped	-/-

berA1.2

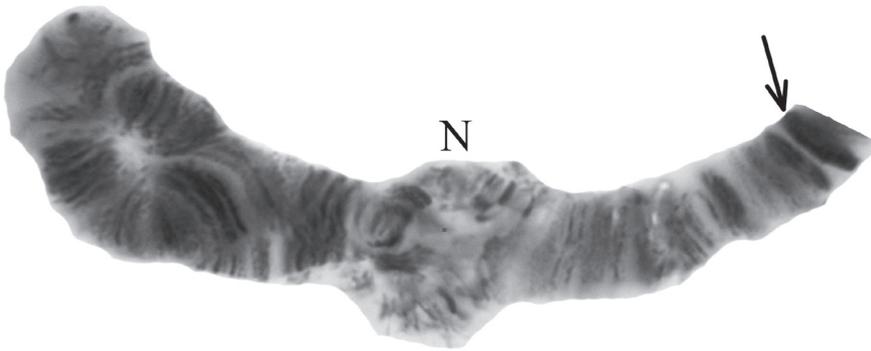


Figure 3. Heterozygous zygotic combination berA1.2. The designations are the same as in Fig. 2.

Table 3. Frequency of banding sequences in different populations of *Ch. bernensis*.

Banding sequence	Populations					
	Western Europe		Central Caucasus			Western Siberia (Istomina and Kiknadze 2004) 60 larvae
	Switzerland (Wülker and Klötzli 1973) 446 larvae	Italy (Petrova and Michailova 2002) 14 larvae	RKB, former riverbed in mouth of Cherek River (original data) 9 larvae	RKB, near Zhemtala village, long-term pool (original data) 39 larvae	KCR, M. Zelenchuk River (original data) 17 larvae	
berA1	0,950	0,821	0,056	0,313	0,411	1,000
berA2	0,050	0,179	0,944	0,687	0,589	-
berB1	1,000	1,000	1,000	1,000	1,000	1,000
berC1	1,000	1,000	0,444	0,700	0,853	1,000
berC2	-	-	0,556	0,300	0,147	-
berD1	1,000	1,000	1,000	1,000	1,000	1,000
berE1	1,000	0,928	0,833	0,975	0,971	0,992
berE2	-	0,036	-	-	-	-
berE3	-	0,036	0,167	0,025	0,029	-
berE4	-	-	-	-	-	0,008
berF1	0,680	abs†	1,000	1,000	1,000	-
berF2	0,320	abs	-	-	-	0,992
berF3	-	0,036	-	-	-	-
berF4	-	-	-	-	-	0,008
berG1	1,000	1,000	1,000	1,000	1,000	0,350
berG2	-	-	-	-	-	0,592
berG3	-	-	-	-	-	0,058
Number of banding sequences in population	9	12	10	10	10	11

†abs - data are absent.

Arm C has two banding sequences – berC1 and berC2. The banding sequence berC1 was dominant in all studied populations (Table 3, 4). The banding sequence berC2 is new for the species and described for the first time (Fig. 4, Table 2–4). It dif-

Table 4. Frequency of zygotic combinations and parameters of chromosomal variability in different populations of *Ch. bernensis*.

Zygotic combinations	Populations					
	Western Europe		Central Caucasus			Western Siberia (Istomina and Kiknadze 2004) 60 larvae
	Switzerland (Wülker and Klötzli 1973) 446 larvae	Italy (Petrova and Michailova 2002) 14 larvae	RKB, former riverbed in mouth of Cherek River (original data) 9 larvae	RKB, near Zhemtala village, long-term pool (original data) 39 larvae	KCR, M. Zelenchuk River (original data) 17 larvae	
berA1.1	0,889	0,643	-	0,025	0,235	1,000
berA1.2	0,101	0,357	0,111	0,617	0,353	-
berA2.2	-	-	0,889	0,358	0,412	-
berB1.1	1,000	1,000	1,000	1,000	1,000	1,000
berC1.1	1,000	1,000	0,111	0,514	0,706	1,000
berC1.2	-	-	0,667	0,358	0,294	-
berC2.2	-	-	0,222	0,128	-	-
berD1.1	1,000	1,000	1,000	1,000	1,000	1,000
berE1.1	1,000	0,857	0,667	0,949	0,928	0,983
berE1.2	-	0,071	-	-	-	-
berE1.3	-	0,071	0,333	0,051	0,072	-
berE1.4	-	-	-	-	-	0,017
berF1.1	0,491	abs†	1,000	1,000	1,000	-
berF2.2	0,130	abs	-	-	-	0,983
berF1.2	0,379	0,357	-	-	-	-
berF2.3	-	0,071	-	-	-	-
berF2.4	-	-	-	-	-	0,017
berG1.1	1,000	1,000	1,000	1,000	1,000	0,150
berG2.2	-	-	-	-	-	0,350
berG1.2	-	-	-	-	-	0,383
berG1.3	-	-	-	-	-	0,017
berG2.3	-	-	-	-	-	0,100
Number of zygotic combinations	10	abs	11	12	11	13
% of heterozygous larva	abs	85,7	78	82,1	59	51,7
Number of heterozygous inversions per specimen	0,480	0,643	1,110	1,000	0,650	0,533
Number of inversions per arm	0,29	0,71	0,43	0,43	0,43	0,71

†abs - data are absent.

fers from berC1 by one simple inversion step that involves regions 4hi-6b 11c-8 15e-b: berC2 1-2c 4hi-6b 11c-8 15e-b 4g-2d 15a-11d 6gh 17a-16 7d-a 6f-c 17b-22 C

The banding sequence berC2 was found in studied populations with high frequency in both homozygous and heterozygous state (Table 3, 4).

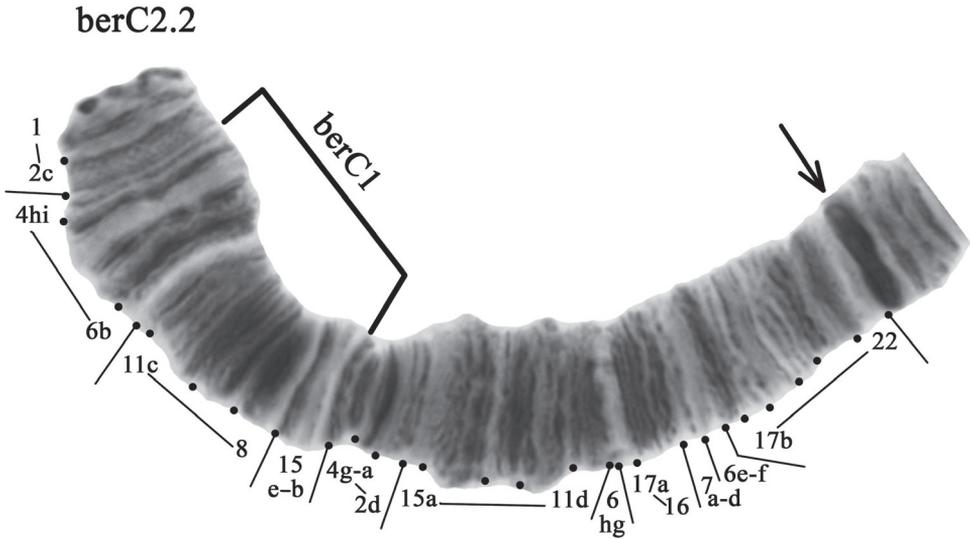


Figure 4. Homozygous zygotic combination berC2.2. The designations are the same as in Fig. 2.

Arm D is monomorphic with banding sequence berD1 found in homozygote state (Fig. 2, Table 2–4).

Arm E had two banding sequences—berE1 and berE3 (Table 2–4). The banding sequence berE1 was dominant in all studied North Caucasian populations (Table 3, 4). The banding sequence berE3 has been found only in heterozygous state (Fig. 5, Table 3, 4).

Arms F and G were monomorphic and presented by sequences berF1 and berG1, respectively (Fig. 2, Table 2–4).

In all three North Caucasian populations the number of banding sequences was identical and equal to 10 (Table 3). The number of zygotic combinations found in studied populations varied from 11 to 12 (Table 4). From 59 to 82% of larvae were heterozygous (Table 4).

In total, 12 genotypic combinations have been found (Table 5). Each studied population was characterized by different dominant genotypic combination. Thus, in RKB (the former riverbed in the mouth of the Cherek River) dominant genotypic combinations were berA2.2B1.1C1.2D1.1E1.1F1.1G1.1 and berA2.2B1.1C1.2D1.1E1.3F1.1G1.1, in RKB (in the vicinity of Zhemtala village, long-term water body) – berA1.2B1.1C1.1D1.1E1.1F1.1G1.1; in KCR (Malyi Zelenchuk River) – berA1.2B1.1C1.1D1.1E1.1F1.1G1.1 and berA2.2B1.1C1.1D1.1E1.1F1.1G1.1.

Comparison of chromosomal polymorphism of *Ch. bernensis* from the Central Caucasus and Ciscaucasia and other parts of the range

As stated above, in all the long polytene chromosomes of *Ch. bernensis* from the studied North Caucasian populations the centromere bands are large and belong to n-type

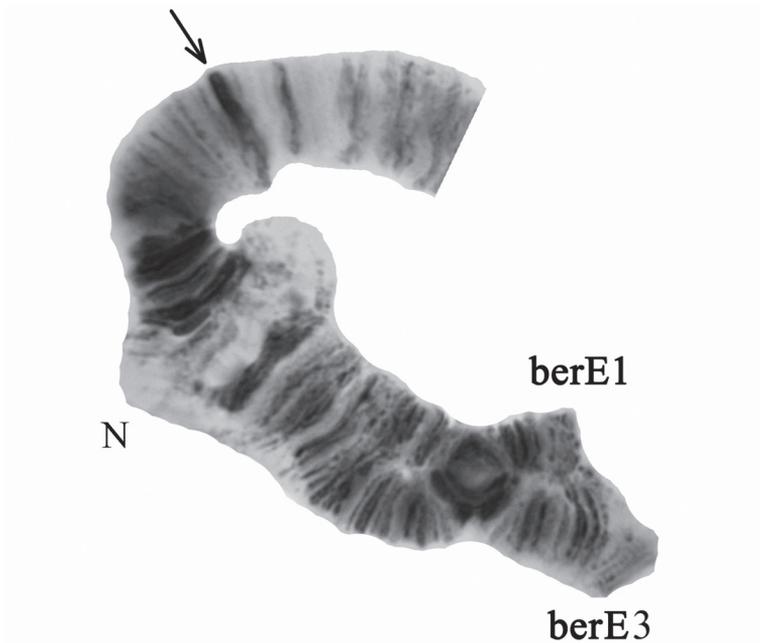


Figure 5. Heterozygote berE1.3 The designations are the same as in Fig. 2.

Table 5. Genotypic combinations *Ch. bernensis* from Central Caucasus and Ciscaucasia.

Genotypic combinations	RKB, former riverbed in mouth of Cherek River (original data) 9 larvae	RKB, near Zhemtala village, long-term pool (original data) 39 larvae	KCR, M. Zelenchuk River (original data) 17 larvae
A1.1B1.1C1.1D1.1E1.1F1.1G1.1	0	0	0,176
A1.1B1.1C1.2D1.1E1.1F1.1G1.1	0	0,025	0
A1.1B1.1C1.1D1.1E1.3F1.1G1.1	0	0	0
A1.1B1.1C2.2D1.1E1.3F1.1G1.1	0	0	0,059
A1.2B1.1C1.1D1.1E1.1F1.1G1.1	0,111	0,308	0,235
A1.2B1.1C1.2D1.1E1.1F1.1G1.1	0	0,128	0,059
A1.2B1.1C1.1D1.1E1.3F1.1G1.1	0	0,025	0,059
A1.2B1.1C2.2D1.1E1.1F1.1G1.1	0	0,103	0
A1.2B1.1C1.1D1.1E1.3F1.1G1.1	0	0	0
A1.2B1.1C1.2D1.1E1.3F1.1G1.1	0	0,025	0
A2.2B1.1C1.1D1.1E1.1F1.1G1.1	0	0,179	0,235
A2.2B1.1C1.2D1.1E1.1F1.1G1.1	0,333	0,179	0,117
A2.2B1.1C1.2D1.1E1.3F1.1G1.1	0,333	0	0
A2.2B1.1C2.2D1.1E1.1F1.1G1.1	0,222	0,025	0
number of genotypic combinations	4	9	7

according to the classification by Shobanov (2002) (Fig. 6). In Siberian populations (Istomina and Kiknadze 2004, Kiknadze et al. 2007) and in the photo of chromosomes in the first description of *Ch. bernensis* from Swiss populations (Wülker and Klötzli

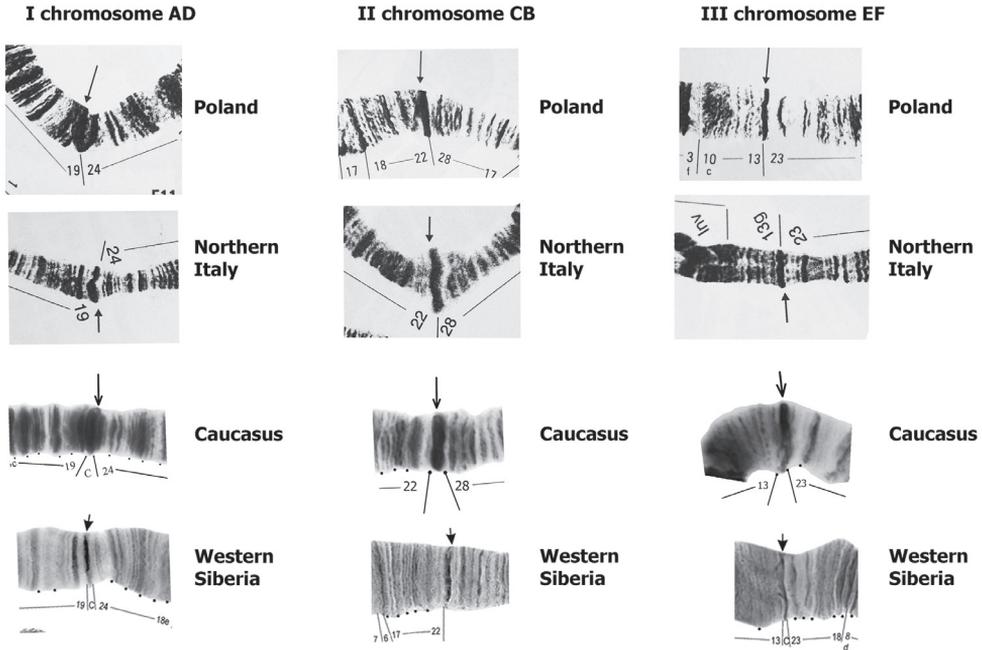


Figure 6. Comparison of pericentromeric regions of polytene chromosomes of *Ch. bernensis* from Caucasian, European and Siberian populations.

1973), the centromere bands are thin and belong to s-type. The large centromeric bands of this species were found in the populations of Bulgaria, Poland, Northern Italy (Michailova 1989, Michailova et al. 2002, Petrova and Michailova 2002).

Data for Polish and Italian populations are presented on the basis of publications of Michailova (1989); Michailova and coauthors (Michailova et al. 2002), Petrova and Michailova (2002), data for Siberian populations are presented on the basis of publications of Istomina and Kiknadze (2004), Kiknadze and coauthors (Kiknadze et al. 2007).

Unfortunately, because of the low number of specimens of *Ch. bernensis* found in most populations of Central Caucasus and Ciscaucasia water bodies studied, only three populations with a significant number of larvae – the former riverbed in the mouth of the Cherek River near Oktyabrskaya village, the long-term water body near Zhemtala village, the backwater in the main riverbed of Malyi Zelenchuk River near Adyl-Khalk village – were used for comparison with populations from other geographic regions (Table 3, 4).

Arm A. The populations from the North Caucasus, as well as populations from Europe–Switzerland (Wülker and Klötzli 1973) and Italy (Petrova and Michailova 2002) – are characterized by the presence of two banding sequences in this arm, berA1 and berA2 (Table 3, 4), whereas only berA1 was present in populations of Western Siberia (Istomina and Kiknadze 2004). At the same time it should be noted that populations from the North Caucasus and Europe differ significantly by the frequencies of banding sequence berA1 and berA2: while the former was dominant in Western

Europe, the latter dominated in North Caucasian populations, occurring there in both the heterozygote and homozygote state.

Arm B and D of *Ch. bernensis* were monomorphic in all studied populations.

Arm C of *Ch. bernensis* were monomorphic in populations from Europe and Siberia but showed high level of inversion polymorphism in studied Caucasian populations due to the presence of a new banding sequence berC2 that might be endemic for this region. However, for *Ch. bernensis* from Spain unmapped chromosomal rearrangement in the arm C was early indicated (Real et al. 2000). The high frequencies of heterozygotes berC1.2 and homozygotes berC2.2 in Caucasian populations (Table 3, 4) clearly distinguishes them from all other populations.

In the **arm E** all studied populations of *Ch. bernensis* share the same dominant banding sequence berE1. At the same time populations from all regions differ from each other by sets of additional banding sequences found in heterozygote state. Thus, in Switzerland this arm was completely monomorphic (Wülker and Klötzli 1973), in Italy two banding sequences – berE2 and berE3 (Petrova and Michailova 2002) – were found with low frequencies in heterozygotes with berE1, while only heterozygotes berE1.3 were found in Caucasian populations and berE1.4 – in populations from Western Siberia (Istomina and Kiknadze 2004). The comparison of the inversion banding sequences of the arm E from different populations shows the most similarity between Caucasian and Italian populations.

Arm F of *Ch. bernensis* in Caucasian populations was monomorphic and presented only by the standard banding sequence berF1 unlike the populations from other regions. In the population of Switzerland (Wülker and Klötzli 1973) the approximately equal number of homo- (ber F1.1) and heterozygotes (ber F1.2) was observed. In the Siberian population banding sequence berF2 was strictly dominant with the only other banding sequence being berF4 that was present with a low frequency in a heterozygote state (berF2.4) (Istomina and Kiknadze 2004), which clearly distinguishes the Siberian population of *Ch. bernensis*.

Arm G of *Ch. bernensis* was monomorphic in both European and Caucasian populations and was presented by the standard banding sequence berG1. At the same time in the Siberian population three banding sequences were found in different zygotic combination (Istomina and Kiknadze 2004) with berG1.2 being the dominant one, which clearly distinguishes this population from the other ones.

Thus, summarizing all data it can be concluded that a significant degree of divergence can be seen between populations of Europe, Caucasus and Western Siberia.

The inversion polymorphism of populations of *Ch. bernensis* from the North Caucasus has much higher level of heterozygous inversions per specimen in comparison with the early studied populations, i.e. 0,65 to 1,11 (Tables 3–5). In the number of genotypic combinations (11), number of banding sequences per population (10) and number of inversions per arm (0,43), the Caucasian populations of this species are intermediate between European (respectively: 10, 9 and 0,29) and Siberian (respectively: 13, 11 and 0,71) populations.

Table 6. Value of cytogenetic distances between the different populations of *Ch. bernensis*.

Population	Switzerland	Italy	RKB (Cherek river)	RKB (Zhemtala)	KCR (M. Zelenchuk River)	Western Siberia
Switzerland	0					
Italy	0,054	0				
RKB (Cherek River)	0,343	0,409	0			
RKB (Zhemtala)	0,176	0,206	0,082	0		
KCR (M. Zelenchuk River)	0,111	0,159	0,092	0,015	0	
Western Siberia	0,130	0,142	0,645	0,424	0,322	0

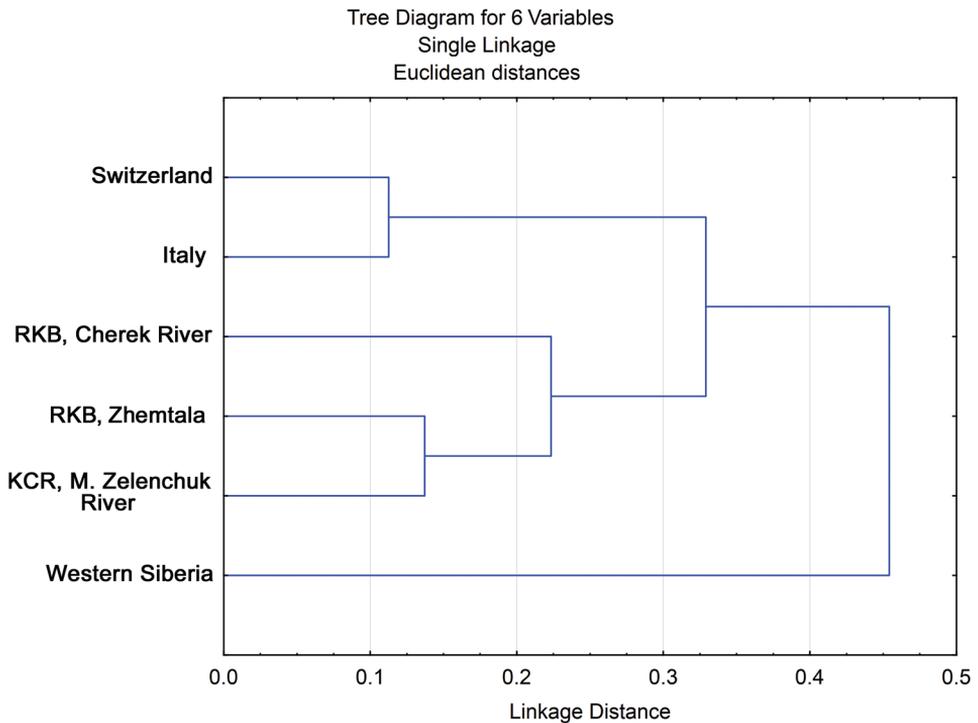


Figure 7. The dendrogram of cytogenetic distances between the samples from different populations of *Ch. bernensis*.

Cytogenetic distances (Table 6), was measured by Nei criteria (1972) on basis of the original data and data of other authors on inversion polymorphism of the species in Europe and Siberia (Fig. 7). These distances indicate the significant distance of the Siberian populations of *Ch. bernensis* and of intermediate position of the Caucasian populations between the populations of Western Europe and Western Siberia.

The dendrogramm was constructed on the basis of Nei criteria (1972) using NJ-method.

In establishing of cytogenetic distances for populations of Siberia, Switzerland and Italy data of other authors were used (Wülker and Klötzli 1973, Petrova and Michailova 2002, Istomina and Kiknadze 2004).

Discussion

In the Central Caucasus (the northern macroslope) and Ciscaucasia *Ch. bernensis* has been found for the first time. At present, 17 banding sequences including berC2 are known in the banding sequences pool of *Ch. bernensis*. The comparative analysis of chromosomal polymorphism between the Caucasian populations and populations of other regions has revealed specific peculiarities: the presence of sequence berA2 in homozygous state, which was not registered in the populations studied earlier, and the presence of banding sequence berC2, which is probably endemic for the region.

The morphological characteristics such as the number of premandible teeth are diagnostic for *Chironomus* species. Thus, among the species of this genus more than two teeth of the premandible can be found in larvae dwelling in the brackish water bodies, i.e. *Ch. behmingi* Goetgh. with five teeth (Pankratova 1983, Polukonova and Beljanina 2002); *Chironomus albidus* Konst. (Konstantinov 1956) and *Ch. sp.* (sibling species of *Chironomus albidus*, apparently belonging to *Ch. paraalbidus* Beljanina et al. 2005a) with three teeth (Polukonova et al. 2004, Beljanina et al. 2005b, Polukonova 2007). It can be suggested that this morphological peculiarity emerged due to such special feature of the chemical composition in water bodies of the Caucasus as increased mineralization. However, such an assertion needs additional research on the water mineralization level in the collection sites of *Ch. bernensis* in the Central Caucasus (the northern macroslope) and Ciscaucasia.

The other significant diagnostic characteristic that allows differentiating the species of genus *Chironomus* is the centromere type (Shobanov 2000, 2002). Thus, several pairs of sibling species with identical banding sequences in the polytene chromosomes (homosequential species), such as *Ch. piger* and *Ch. riparius* (Keyl and Strenzke 1965, Polukonova et al. 1996, Karmokov et al. 2011) or *Ch. nuditarsis* and *Ch. curabilis* (Polukonova et al. 2003, 2005, Polukonova 2005a, b), were found to be different in the size of the pericentromeric heterochromatin. Although it is necessary to note that intra- and interpopulation chromosomal polymorphism can be observed for this characteristic (Iliyinskaya 1984, Kiknadze and Siirin 1991, Kiknadze et al. 1991b), which can complicate its use as a species-specific criteria especially in the cases when the difference in centromere size of different species is not very significant.

The dominance of different genotypic combinations at various sites of the Caucasus probably can be explained by the fact that in some areas some combinations can be more adaptive than the others. Perhaps this is happening due to a different level of mineralization, temperature and degree of eutrophication in the different collection sites.

Caucasian populations on the dendrogram occupy an intermediate position between Italian and Swiss populations, on the one hand, and Western Siberian popula-

tion, on the other. Such arrangement agrees rather well with the geographic location of the studied regions and may reflect the true course of settlement of the species (either from west to east or from east to west). For more specific allegations more researches are needed.

In the context of the data mentioned above, further researches on *Ch. bernensis* from geographically distant regions are necessary, as there is possibility that the presently known species is actually polytypic and consists of several sibling species.

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Genetic integrity of four species of *Leptidea* (Pieridae, Lepidoptera) as sampled in sympatry in West Siberia

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Abstract

In southern West Siberia, as many as four *Leptidea* Billberg, 1820 species are present sympatrically: *L. amurensis* (Ménétriés, 1859), *L. morsei* (Ménétriés, 1859), *L. sinapis* (Linnaeus, 1758) and *L. juvernica* Williams, 1946. The two latter were recently recognised as nearly sibling species on morphological and molecular characters. Specimens intermediate as to their subtle diagnostic characters occurring in West Siberia and elsewhere were interpreted as resulted from limited introgression. This supposition was tested via populational morphological and molecular analysis of spring brood specimens of all the four species taken from a limited (4.5 × 0.2 km) area in the suburbs of Novosibirsk. The samples were analysed with respect to the genitalic morphology, external characters, three nuclear (*CAD*, *H1* gene and *ITS2*) and one mitochondrial (*COI*) molecular markers, infection of the intracellular maternally inherited bacterial symbiont *Wolbachia* Hertig, 1836 and its *wsp* gene coding for a hypervariable surface protein. Interspecific variation of the nuclear *CAD* and *ITS2* sequences and the mitochondrial *COI* gene in *L. sinapis* and *L. juvernica* turned out concordant. The absence of molecular evidence of introgression suggests genetic integrity of these two species and allows their reliable identification by molecular characters. The genitalic (lengths of the saccus and valva) and external characters (wing pattern) of males overlap in *L. sinapis* and *L. juvernica*, as identified by molecular markers and thus are not so helpful in actual species identification. Only the ductus bursae length showed no overlap and can be used for identification of females. The histone H1 gene appeared five times less variable over the four studied species than *COI*, and found to be identical in species *L. sinapis* and *L. juvernica*. *Wolbachia* infection was found in all studied species. We identified three *wsp* variants of *Wolbachia*: 1) *wsp-10* allele in *L. amurensis*, *L. sinapis*, *L. juvernica*; 2) a very similar *wsp-687* allele in *L. sinapis*; and 3) *wsp-688*, highly divergent to the previous ones, in *L. morsei*.

Keywords

Leptidea, Lepidoptera, *Wolbachia*, introgression, molecular markers, histone H1, *COI*, *ITS2*, *wsp*, genitalia morphology, intraspecific variation

Introduction

The genus *Leptidea* Billberg, 1820 (Dismorphiinae, Pieridae) includes several (at least eight) Palearctic species. Recently it attracted attention because of repeated and rather unexpected discoveries of sibling species (Dincă et al. 2011). Firstly, *Leptidea lorkovicii* Réal, 1988 was separated from the sympatric *L. sinapis* (Linnaeus, 1758) on the basis of substantial differences in the genitalia structure (Réal 1988). Later a new name *Leptidea reali* Reissinger, (1990) was proposed to it because of existence of a senior homonym *Leptidea duponcheli lorkovici* Pfeiffer, 1932 (Reissinger 1990). Note that later it was found out (Dincă et al. 2011) that the new name was not necessary and invalid because of existence of an older available name *juvernica* Williams, 1946 proposed for an Irish population showing the relevant morphology. Secondly, on the basis of molecular and karyological data, the species known under the invalid name *L. reali* was split into two allopatric species, *L. reali* s. str. from Spain, southern France and Italy (for this species the name in narrow sense is valid) and *Leptidea juvernica* Williams, 1946 ranging from the French Pyrenees in the south-west and Ireland in the north-west to Central Siberia in the east (Dincă et al. 2011). Citing literature data *de facto* dealing with *L. juvernica*, below we will use this name although before 2011 the authors used the name *L. reali*. Ranges of both *L. reali* and *L. juvernica* overlap with that of *L. sinapis* ranging from Spain and Ireland to East Siberia (Dincă et al. 2011). Hence *L. sinapis* and *L. juvernica* co-occur on a vast territory from South Europe to Central Siberia.

The main diagnostic character of *L. reali* and *L. juvernica* versus *L. sinapis* is a substantially greater relative lengths of the aedeagus and saccus (which correlate to each other) in the male genitalia and of the ductus bursae in the female genitalia (Fumi 2008, Ivonin et al. 2009, Dincă et al. 2011, Sachanowicz 2013). There are less distinct differences between *L. juvernica* and *L. sinapis* in the wing shape and pattern. For both the European part of Russia (Bolshakov 2005, Bolshakov et al. 2013) and West Siberia (Ivonin et al. 2009), the following differences between *L. juvernica* and *L. sinapis* in the wing coloration were claimed:

- (i) spring brood males of *L. juvernica* have in general darker, more suffused hind wing underside below vein M3 and with less distinct stripy pattern (less expressed lighter postdiscal spots between veins) than those of *L. sinapis*;
- (ii) summer brood males of *L. juvernica* differ from those of *L. sinapis* in the fore wing upperside without the light rim along the apical dark spot and darkened ends of veins M3 and Cu1;
- (iii) in West Siberia, the spring brood males of *L. juvernica* were claimed to have a more attenuated fore wing apex than those of *L. sinapis* (Ivonin et al. 2009).

No external differences were revealed between females of the two species.

Authors working in different regions (Verovnik and Glogovčan 2007, Ivonin et al. 2009, Bolshakov et al. 2013) pointed out a substantial variation in these two species. Several morphs were recognised based on the tint of wing coloration, which are shared by both *L. sinapis* and *L. juvernica* (Bolshakov et al. 2013). The lengths of the aedeagus and saccus taken alone do not allow distinguishing *L. sinapis* from *L. juvernica* in all cases because of some overlap in *L. sinapis* and *L. juvernica* (Fumi 2008, Sachanowicz 2013). Tsvetkov (2007) reported that samples of *L. juvernica* from forested and open habitats differed in the average relative aedeagus length, which is less in forested habitats. It was even hypothesized that such variation could be supported by selection for a longer aedeagus to ensure mating in more windy open habitats (Bolshakov et al. 2013); however, this would demand a long lasting maintenance of genetic isolation between the two habitat types, which hardly exists at all. Bolshakov et al. (2013) reported specimens with intermediate genitalia from Mordovia Republic (European Russia), e.g. males of *L. juvernica* with a normally long aedeagus but the saccus short as in *sinapis* and curved as in *juvernica*. Ivonin et al. (2009) reported the occurrence in Novosibirsk Province (West Siberia, Russia) of external characters of *L. juvernica* among males with the genitalia of *L. sinapis* (with a short and straight saccus) but not vice versa, for no external characters of *L. sinapis* were found in males with the genitalia of *L. juvernica* (with a long and S-like curved saccus). Verovnik and Glogovčan (2007) reported the occurrence of males that were intermediate between *L. sinapis* and *L. juvernica* in Slovenia, namely: (i) with the aedeagus of intermediate length; (ii) with long saccus but short aedeagus and (iii) with the genitalia of *L. juvernica* but closer to *L. sinapis* according to RAPD markers.

These facts can be interpreted in three ways: (i) as resulting from some gene exchange (introgression) between *L. sinapis* and *L. juvernica*; (ii) as common polymorphism of genes affecting the genitalia structure and/or wing coloration, inherited from the common ancestor, or (iii) by independent mutations (homoplasy) of these hypothetical genes.

The relationships of closely related species may be clarified via two approaches, the phylogeographic and population genetic ones. The former approach implies accumulation of data from a territory as broad as possible in order to reconstruct the history of divergence and spread of species. The latter approach consists of analysing large samples from certain populations in order to register phenomena such as deviations from panmixia, linkage disequilibrium, gene exchange between sympatric taxa, and effects of natural selection.

Relationships between sibling species of *Leptidea* were mostly studied via the phylogeographic approach applied to the entire species ranges (Lukhtanov et al. 2011, Dincă et al. 2011, 2013) using the mitochondrial *COI* and *ND1* genes, the nuclear *CAD*, *ITS2*, and *Wg* markers, and karyotype. These studies did not reveal any introgression between *L. reali*, *L. juvernica* and *L. sinapis* (Lukhtanov et al. 2011, Dincă et al. 2011, 2013). Examples of population genetic studies are the analysis of several sympatric populations of *L. reali* and *L. sinapis* in the French Pyrenees (Martin et al. 2003) and of *L. juvernica* and *L. sinapis* in Slovenia (Verovnik and

Glogovčan 2007). Martin et al. (2003) rejected the introgression hypothesis while Verovnik and Glogovčan (2007) did not exclude some degree of gene exchange between *L. sinapis* and *L. juvernica*.

Another approach is searching for particular mechanisms of isolation between these two species. Hybridisation experiments revealed that prezygotic isolation between *L. sinapis* and *L. juvernica* or *L. reali* was probably based on behavioral barriers, for instance recognition by females of a species-specific courtship behaviour of males or species-specific pheromones (Friberg et al. 2008b, Dincă et al. 2013). Non-conspecific matings between these species were not observed while conspecific individuals from geographically remote populations mated successfully (Dincă et al. 2013). A shifted flight period and some habitat segregation also contribute to the prezygotic isolation between *L. sinapis* and *L. juvernica* (Friberg et al. 2008a). Differences in larval food-plant species were not found (Friberg and Wiklund 2009).

The western foothills of the Altay-Sayan Mountain System (West Siberia, Russia) are unique in being inhabited by four *Leptidea* species altogether, more than elsewhere in the world: *Leptidea morsei* (Fenton, 1881), *L. amurensis* (Ménétriés, 1859), *L. sinapis* and *L. juvernica* (Fig. 1). They have different habitat preferences: at least in Novosibirsk Province: *L. morsei* mostly inhabits open woods, *L. sinapis* — various meadows, *L. amurensis* and *L. juvernica* mostly inhabit meadow steppes in rough relief terrains (Ivonin et al. 2009). In spite of these preferences, all the four species coexist with nearly equal abundance and similar flight period on grassy glades on the eastern bank of the Novosibirsk Water Reserve in the Novosibirsk Academy Town (Kosterin et al. 2007), making this territory an excellent site for studying isolation *vs* introgression of *Leptidea* spp. Hence, we attempted a pure population genetic approach and analysed a sample of spring brood specimens of *Leptidea* collected from the same small area at the junction of the Novosibirsk city and Berdsk town.

The main attention was paid to the closely related and supposedly hybridising species *L. sinapis* and *L. juvernica*. They were analysed with respect to the popular mitochondrial marker *COI* (the gene for cytochrome oxidase I) and the nuclear markers *CAD* (the gene for carbamoyl phosphate synthetase II, aspartate carbamoyltransferase, dihydroorotase), *ITS2* (internal transcribed spacer 2 in the ribosome cluster), and the histone H1 gene, designated here as *H1*. A histone H1 gene was recently proposed as a good phylogenetic marker (Zaytseva et al. 2012, 2015, Solovyev et al. 2015). We also analysed infection by the maternally inherited endosymbiont *Wolbachia* Hertig, 1836 (Zhou et al. 1998; Van Meer et al. 1999; Baldo and Werren 2007), and sequences of its highly variable gene *wsp* (*Wolbachia* surface protein). In addition, the males of *L. sinapis* and *L. juvernica* were analysed for the lengths of the valve and saccus in the male genitalia and scored for the wing characters, and the ductus bursae was measured in females. The *COI*, histone *H1* and *wsp* genes were also sequenced and the *Wolbachia* infection was assessed in the two other co-occurring species, *L. morsei* and *L. amurensis*. Since their external characters are constant and sufficient for reliable identification, their genitalia were not examined.

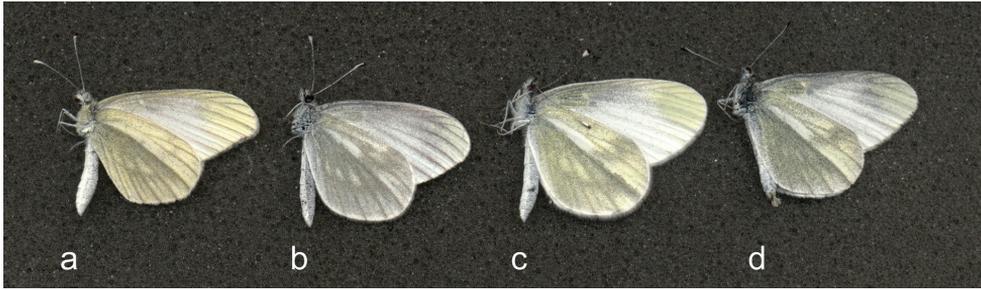


Figure 1. Spring brood males of four species of *Leptidea* Billberg, 1820: *L. amurensis* (Ménétriés, 1859) (a), *L. morsei* (Fenton, 1881) (b), *L. sinapis* (Linnaeus, 1758) (c) and *L. juvernica* Williams, 1946 (d), simultaneously collected in the studied area at the border of Novosibirsk city and Berdsk town, West Siberia, Russia (after Ivonin et al. 2009). Note the difference in the shape of the fore wing apex.

Materials and methods

Material

Seventy spring brood specimens of *Leptidea* spp. were collected in the vicinity of Novosibirsk Academy Town, Novosibirsk Province. The collection area was a 100–200 m wide and a 4.5 km long continuous stripe of meadows adjacent to birch/ pine forests, along the bank of the Novosibirsk Water Reserve and the parallel railroad, between Obskoe More railway station (54°47'37"N; 83°04'34"E; (DMS)) and a point (54°50'04"N; 83°04'40"E (DMS)), 900 m NNE of Rechkunovka railway station, at elevations of 107–137 m a.s.l. (see the locality on a schematic map of northern Eurasia in Fig. 2). Note that the northern half of this area belongs to the city of Novosibirsk while the southern half to the satellite town of Berdsk (with the border at Beregovaya railway station in the middle of the collecting area). Berdsk is the type locality of the subspecies *Leptidea reali yakovlevi* Mazel, 2003 (the justification of which to our opinion have been insufficiently reasoned in the original description). The specimens were collected by O.E. Kosterin in June 2010, May 2011 and May 2012 with a net and frozen immediately. Details of the specimens examined are provided in Table 1. In screening for *Wolbachia* infection, the combined sample of *L. juvernica* and *L. sinapis* was updated with 15 more specimens which were not identified to either of these two species, not analysed in other respects and not included into Table 1, so that the total sample of *Wolbachia* screening contained 85 *Leptidea* specimens.

DNA extraction

Genomic DNA was extracted according to Bogdanova et al. (2009), with modifications for isolation from individual insects. Frozen specimens without abdomen and wings were homogenized in 0.6 ml 0.15 M NaCl. The homogenate was centrifuged

Table 1. Material collected, *COI* gene allelic states as revealed by CAPS approach (denoted as follows: s – *Leptidea sinapis*, j – *L. juvernica*, a – *L. amurensis*, m – *L. morsei*), European Nucleotide Archive (ENA) accession numbers of the *COI* and *H1* gene sequences, presence of *Wolbachia* infection (+ detected; - not detected) and the *wsp* alleles according to the PubMLST database.

Specimen	Sex	Date collection	<i>COI</i> variant	<i>COI</i> ENA accession number	<i>H1</i> ENA accession number	<i>Wolbachia</i> infection (<i>wsp</i> allele)
L1	♂	05.06.2010	s			-
L2	♂	05.06.2010	j			+
L3	♂	05.06.2010	j			+
L4	♂	05.06.2010	s			+
L5	♂	05.06.2010	j			+
L6	♂	05.06.2010	s			+
L7	♂	06.06.2010	s			+
L8	♂	06.06.2010	j			+
L9	♂	06.06.2010	j			+
L10	♂	05.06.2010	j	HG969218	LN606440	+
L11	♂	29.05.2011	j	HG969219		+
L12	♂	29.05.2011	j	HG969220	LN606441	+ (<i>wsp</i> -10)
L13	♂	29.05.2011	j	HG969221		+
L14	♂	29.05.2011	j	HG969222		+
L15	♂	29.05.2011	s	HG969223	LN606442	+
L16	♂	29.05.2011	s	HG969224	LN606443	+ (<i>wsp</i> -687)
L17	♂	29.05.2011	s	HG969225	LN606444	+ (<i>wsp</i> -10)
L18	♂	29.05.2011	s	HG969226		+
L19	♂	13.05.2012	j	HG969227		+
L20	♂	13.05.2012	j			+
L21	♂	13.05.2012	j			+
L22	♂	13.05.2012	s			+
L23	♂	13.05.2012	s			+
L24	♂	14.05.2012	j			+
L25	♂	14.05.2012	s			+
L26	♂	15.05.2012	s			-
L27	♂	15.05.2012	j			+
L28	♂	15.05.2012	j			+
L29	♀	29.05.2010	j			+
L30	♀	05.06.2010	j			+
L31	♀	05.06.2010	j			+
L32	♀	05.06.2010	j			+
L33	♀	05.06.2010	j			+

Specimen	Sex	Date collection	COI variant	COI ENA accession number	H1 ENA accession number	<i>Wolbachia</i> infection (<i>wsp</i> allele)
L34	♀	05.06.2010	s			-
L35	♀	05.06.2010	j			+
L36	♀	06.06.2010	j			+
L37	♀	06.06.2010	s			+
L38	♀	06.06.2010	j			+
L39	♀	13.05.2012	j			+
L40	♀	13.05.2012	j			+
L41	♀	13.05.2012	j			+
L42	♀	13.05.2012	j			+
L43	♀	14.05.2012	s			+
L44	♀	14.05.2012	s			+
L45	♀	14.05.2012	j			+
L46	♀	14.05.2012	s			+
L47	♂	29.05.2010	a	HG969228		+
L48	♂	29.05.2010	a	HG969229		+
L49	♂	26.05.2011	a	HG969230		+
L50	♂	26.05.2011	a	HG969231	LN606445	+
L51	♂	29.05.2011	a	HG969232		+
L52	♂	29.05.2011	a	HG969233		+
L53	♂	29.05.2011	a	HG969234		+
L54	♀	28.05.2010	a	HG969235		+
L55	♀	28.05.2010	a	HG969236		+
L56	♀	26.05.2011	a	HG969237		+
L57	♀	26.05.2011	a	HG969238		+
L58	♀	26.05.2011	a	HG969239	LN606446	+ (<i>wsp</i> -10)
L59	♀	29.05.2011	a	HG969240		+
L60	♂	29.05.2010	m	HG969241	LN606447	+
L61	♂	29.05.2011	m	HG969242		+
L62	♂	29.05.2011	m	HG969243		+
L63	♂	29.05.2011	m	HG969244		+
L64	♂	29.05.2011	m	HG969245		+
L65	♂	29.05.2011	m	HG969246		+
L66	♀	29.05.2010	m	HG969247		+
L67	♀	26.05.2011	m	HG969248	LN606448	+ (<i>wsp</i> -686)
L68	♀	29.05.2011	m	HG969249		+
L69	♀	29.05.2011	m	HG969250		+
L70	♀	29.05.2011	m	HG969251		+

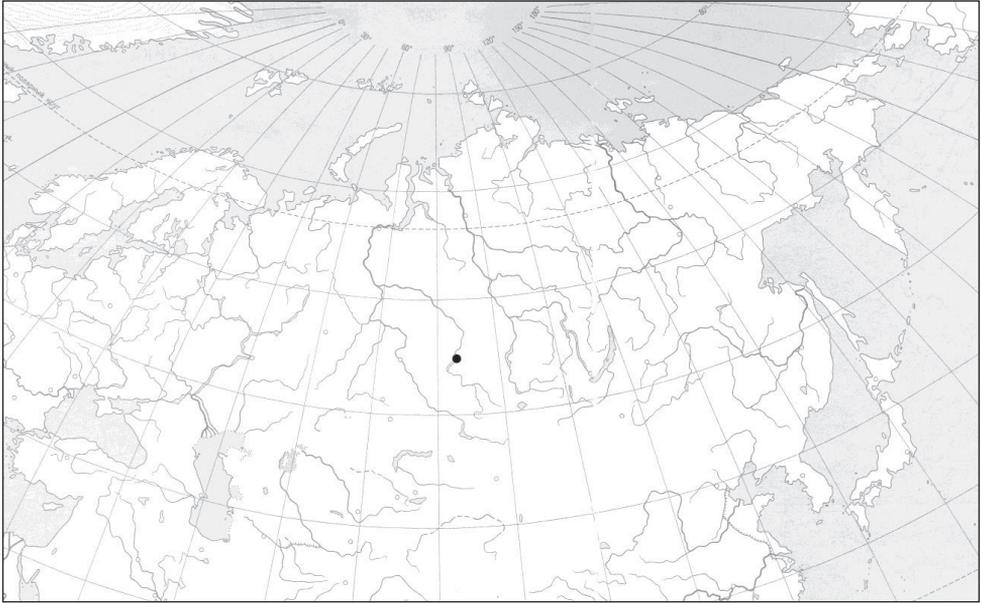


Figure 2. Position (black circle) of the studied area at the border of Novosibirsk city and Berdsk town ($54^{\circ}47'37''\text{N}$; $83^{\circ}04'34''\text{E}$ – $54^{\circ}50'04''\text{N}$; $83^{\circ}04'40''\text{E}$; DMS), Novosibirsk Province, Russia, on a schematic map of northern Eurasia.

(3,300 rcf, 5 min) and the supernatant was discarded, then 0.2 ml solution for DNA extraction (0.1 Tris-HCl, pH 8.0; 5 mM EDTA; 0.5% SDS; 0.1 M NaCl) was added and incubated at room temperature for 40 minutes. Then the solution was centrifuged (16,100 rcf, 5 min) and the pellet was discarded. To remove proteins and RNA, LiCl (0.2 ml, 5M) was added to the supernatant solution and incubated on ice for 15 min. The solution was centrifuged (16,100 rcf, 5 min) and the supernatant was transferred to fresh tubes. Ethanol (1 ml, 96%) was added and the mixture was incubated on ice for an hour. Then it was centrifuged (16,100 rcf, 10 min) and the supernatant was discarded. The precipitate was washed with 0.1 ml 75% ethanol and centrifuged (16,100 rcf, 5 min), then dried at 50°C for 5 min and dissolved in 50 μl of deionized H_2O .

DNA amplification and sequencing

A 708 bp long fragment of the *COI* gene, positions 1526–2156 (positions are given according to the mitochondrial reference of *Drosophila yakuba* Burla, 1954 (AN X03240)), was amplified with the universal insect primers LCO-1490 and HCO-2198 (Folmer et al. 1994). A 684 bp sequence of *ITS2* (internal transcribed spacer 2) and 571 bp sequence of *CAD* (carbamoyl phosphate synthase II, Aspartate carbamoyl-transferase, dihydroorotase) were amplified with primer pairs ITS3/ITS4 and CADFa/CADRa, respectively, following Dincă et al. (2011). The *H1* gene was amplified with

primers designed for two overlapping sequences: the 5' terminal part with the primer pair LH4-f (5'ACCCTGTACGGTTTCGGCGGTTAA) and HeH1-r (5' AGCGC-CCTTGCCCTTGGTCTGTATC) and the 3' terminal part of gene with another pair, HeH1-f (5'ACCCACCCCAAGACCTCCGAGATGGT) and LeH1C-r (5'AGGGG-GACTCACTTTTGGGA). The 5' terminal fragment is approximately 1.5 kbp long, and the 3'-terminal fragment is 650 bp long. The primers were originally designed to match orthologous sequences of *Bombyx mori* (Linnaeus, 1758) (LH4-f), *Heliconius erato* (Linnaeus, 1758) (HeH1-r and HeH1-f) (Solovyev et al. 2015) and *L. sinapis* (LeH1C-r); they were produced by Biosset (Novosibirsk, Russia).

DNA samples were examined for *Wolbachia* infection by amplification of *wsp* with the following primer set: *wsp*81F (5'TGGTCCAATAAGTGATGAAGAAAC-3'), *wsp*691R (5'AAAAATTAAACGCTACTCCA-3') (Braig et al. 1998). PCR products of five DNA stocks of four species were sequenced.

PCR mixtures (30 µl) contained 0.2 mM of each dNTP, 1.5 mM MgCl₂, 25mM KCl, 60 mM Tris-HCl (pH 8.5), 10 mM β-mercaptoethanol, 0.1% Triton X-100, 0.5 µM of each primer, 1 µl of genomic DNA solution and 1 U of Taq DNA polymerase or 1 U of Smart-Taq DNA Polymerase (by Laboratory Medigen, Novosibirsk, Russia). PCR was performed using a thermal cycler MyCycler (Bio-Rad, USA) with the following program: 1) 94 °C — 2 min 30 s, 1 cycle; 2) 95 °C — 15 s, 47–55 °C — 30 s, 68 °C — 1 min, 35 cycles; 3) 68 °C — 2 min, 1 cycle.

The entire coding sequence of *H1* and a 631 bp long fragment of *COI* (positions 1526–2156) were sequenced. The Sanger reaction was conducted in 30 µl volume of mixture containing 1 µl of BigDye Terminator, version 3.1 (Applied Biosystems), 100–200 ng of DNA, 3 pmol of primer and 6 µl of buffer solution for BigDye 3.1. A MyCycler (Biorad) thermocycler was used with the following program: 95 °C — 45 s, 50 °C — 30 s, 60 °C — 4 min; 26 cycles. Sequencing was made at the SB RAS Genomic Core Facility, Novosibirsk.

Sequence alignments and calculation of the genetic distances were performed using the MEGA 5.0 software package (Tamura et al. 2011).

CAPS genotyping *L. sinapis* and *L. juvernica*

For genotyping the *L. sinapis* and *L. juvernica* specimens with respect to certain diagnostic nucleotide substitutions in mitochondrial and nuclear markers, CAPS analysis was conducted (Konieczny and Ausubel 1993). After the analysis of DNA sequences of *L. sinapis* and *L. juvernica* in public databases, we picked the set of endonucleases *Hpa*II, *Alu*I and *Hind*III for genotyping the *COI* gene, *ITS2* region and *CAD* gene, respectively.

The 708 bp long amplified fragment of *COI* of *L. juvernica* contains three restriction sites for endonuclease *Hpa*II and is digested to 4 fragments (66, 109, 206, 327 bp), while the orthologous fragment of *L. sinapis* has no restriction sites. The *ITS2* region of *L. sinapis* contains the only site specific for endonuclease *Alu*I and is digested into 2 fragments (412, 272 bp); the *ITS2* of *L. juvernica* does not contain restriction

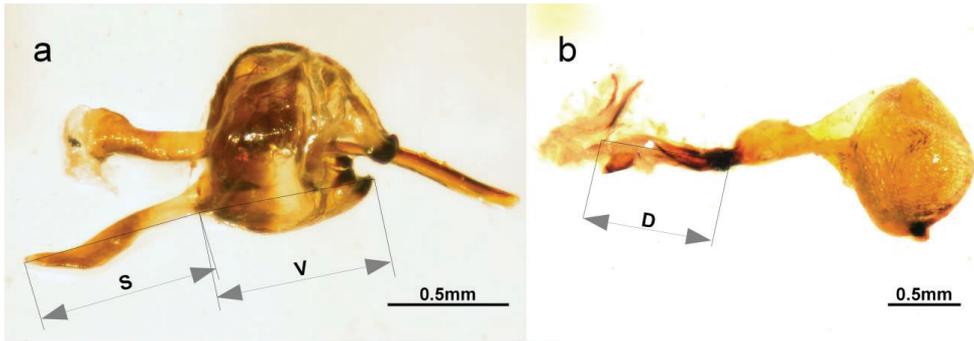


Figure 3. Male (a specimen L12) and female (b specimen L45) genitalia of *Leptidea juvernica*, with measured parameters indicated, namely the length of the saccus (S), valve (V) and ductus bursae (D).

sites for *AluI*. The *CAD* sequence of *L. juvernica* includes only one restriction site for endonuclease *HindIII*, which digests it into two fragments, 110 and 461 bp in length; the *CAD* sequence of *L. sinapis* has two sites which produce three digestion fragments (110, 189, 272 bp). The buffers and enzymes for restriction reactions were produced by Sibenzim, Novosibirsk, Russia. The identical procedure was used for different markers, as follows: 9 μ l of the PCR product was added with 0.5 U of endonuclease and 1 μ l of a buffer relevant to the endonuclease. The mixture was incubated at 37 °C for 2 hr, inactivated at 80 °C for 20 min and analyzed by electrophoresis in 1.5% agarose.

Genitalia morphometrics

The abdomen tip with the genitalia was taken from frozen specimens of *L. juvernica* and *L. sinapis*, incubated for 10 min at 98 °C in 10% potassium hydroxide for maceration and dissected under a stereomicroscope. Lengths of the valve (V) and saccus (S) were measured with an ocular-micrometer and binocular lens MBS-2, as shown in Fig. 3. Besides, the saccus curvature was classified as referring to arbitrary binary scores: 0 – straight, 1 – S-like curved.

Statistical analyses were carried out using MS Excel 10 for Windows.

The genitalia were analysed before molecular analysis, which was carried out blindly of the genitalic results. The specimens in which molecular results appeared discordant with morphological ones, were then rechecked for morphology and discordancy was confirmed.

External characters

The external characters reported to be different in the spring brood males of *L. juvernica* and *L. sinapis*, namely (i) the wing underside below vein M3 more suffused by dark scales and with less expressed lighter spots between veins and (ii) more attenuate fore

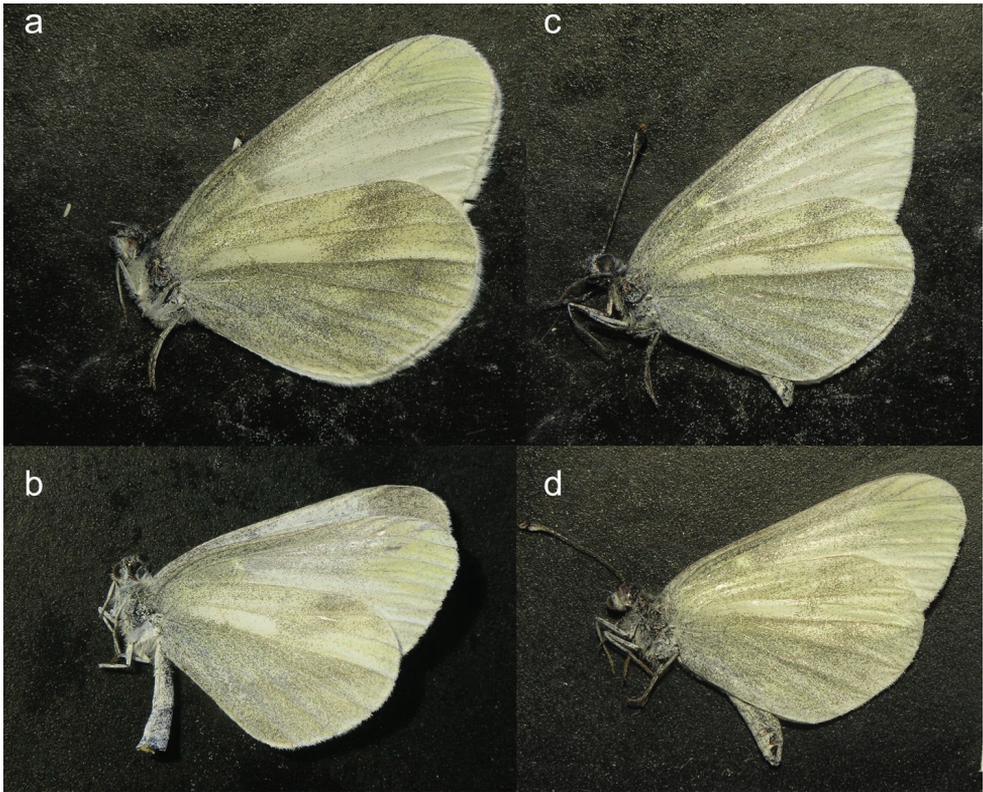


Figure 4. Males of *Leptidea sinapis* (**a** specimen L1 **b** specimen L26) and *Leptidea juvernica*, (**c** specimen L3 **d** specimen L8), with different scores of subjectively evaluated wing characters: the hind wing underside suffusion below vein M3: 0 – lighter, with better expressed lighter spots between veins; 1 – stronger, with scarcely seen lighter spots; and the fore wing apex shape: 1 – more acute, 0 – more rounded. The scores for the shown specimens are as follows (suffusion, apex shape): **a** (0,0); **b** (1,0); **c** (1,1); **d** (1,0); **a** and **c** are variants most frequent in the respective species.

wing apex in the former species (Ivonin et al. 2009), are difficult to measure and somewhat subjective. Therefore we classified them as referring to arbitrary binary classes:

- hind wing underside suffusion below vein M3: 0 – with well-expressed lighter spots between veins, 1 – stronger, more even, with scarcely or expressed lighter spots;
- fore wing apex: 0 – broadly rounded; 1 – more attenuated and acute (Fig. 4).

Results

Inter- and intraspecies variation of mitochondrial *COI* gene

The 631 bp long fragment of the mitochondrial gene *COI* (position 1526 – 2156) was sequenced for 34 *Leptidea* specimens (10 of *L. sinapis* + *L. juvernica*, 11 of *L.*

Table 2. Polymorphic positions in the *COI* gene in *Leptidea sinapis* (specimens L15-L18) and *L. juvernica* (specimens L10–L14 and L19). Positions discriminating *s*- and *j*- allele types are boldfaced; intraspecific substitutions are underlined.

Specimens representing six alleles		L10, L11, L14	L12, L13	L19	L15	L16, L17	L18
Position	allele type	j	j	j	s	s	s
	1530	T	T	T	C	C	C
	1587	A	A	A	<u>A</u>	G	G
	1599	C	C	C	T	T	T
	1615	<u>A</u>	G	G	G	G	G
	1624	A	A	A	G	G	G
	1659	T	T	T	C	C	C
	1674	G	G	G	<u>G</u>	A	A
	1686	<u>T</u>	C	C	C	C	C
	1720	C	C	C	T	T	T
	1854	C	C	C	T	T	T
	1860	A	A	A	G	G	G
	1914	T	T	T	C	C	C
	1917	C	C	<u>T</u>	T	T	T
	1926	C	C	C	T	T	T
	1947	A	A	A	G	G	G
	1959	C	C	C	T	T	T
	2076	T	T	T	T	T	<u>A</u>
	2103	C	C	C	T	T	T
	2121	T	T	T	A	A	A
2133	G	G	G	A	A	A	
2148	C	C	C	T	T	T	

morsei and 13 of *L. amurensis*) collected in the same locality. The sequences were submitted to European Nucleotide Archive (ENA), for accession numbers see Table 1. The sequences of *L. morsei* specimens were identical. In *L. amurensis*, two alleles were found which differed in position 1969, occupied by either T or A. The 1969T allele was found in 12 specimens while the 1969A allele was only found in only specimen L57. Six *COI* alleles were revealed in *L. sinapis* and *L. juvernica*. These six alleles differed in 22 sites (Table 2) and formed two groups of three alleles each, further referred to as the *s*- and *j*-alleles. The consensuses of each group differed in 17 substitutions, the other 5 substitutions were not diagnostic. The *j*-alleles differed from each other in substitutions in the positions 1615, 1686, 1917. Two of the *s*-alleles differed in T/A substitution in position 2076, while the third, found in specimen L15, has positions 1587 and 1674 occupied by the nucleotides otherwise specific for *j*-alleles. Hence the two latter positions were not diagnostic. As a result, the set of positions diagnostic for the *s*- and *j*-type, which allows species identification, included 15 positions (Table 2).

Table 3. Evolutionary Divergence over Sequence Pairs between *Leptidea* species in the studied sample as calculated from 34 *COI* gene sequences obtained. The number of nucleotide substitutions per site averaged over all possible specimens pairs for any two species, \pm its standard error, is shown below the main diagonal, their minimum value among all specimens pairs for any two species is shown above the main diagonal. The total number of positions was 631.

	1	2	3	4
1. <i>L. juvernica</i>		0.024	0.041	0.052
2. <i>L. sinapis</i>	0.029 \pm 0.006		0.043	0.052
3. <i>L. morsei</i>	0.043 \pm 0.008	0.043 \pm 0.008		0.048
4. <i>L. amurensis</i>	0.055 \pm 0.008	0.053 \pm 0.008	0.048 \pm 0.008	

The averaged and minimum *p*-distances between of the studied *COI* fragment between *L. juvernica* (*j*-alleles), *L. sinapis* (*s*-alleles) *L. morsei*, *L. amurensis*, of are provided in Table 3.

The *wsp* gene variation

PCR amplification of the *wsp* gene revealed *Wolbachia* infection in 38 of 42 tested males and 18 of 19 tested females of the *L. sinapis* + *L. juvernica* united sample (91.8% prevalence), in all 11 tested specimens of *L. morsei* and in all 13 tested specimens of *L. amurensis* (100% prevalence) (Table 1, but 15 specimens of *L. sinapis* or *L. juvernica*, analysed only for *wsp*, are not included into the table).

The *wsp* gene was sequenced in one specimen of each *L. amurensis* (L58), *L. morsei* (L67) and *L. juvernica* (L12) and two specimens of *L. sinapis* (L16, L17). The sequences were submitted to the PubMLST database <http://pubmlst.org> [accessed 30 January 2015] (for accession numbers see Table 1). *L. amurensis*, *L. juvernica* and one specimen (L17) of *L. sinapis* turned out to have allele *wsp*-10, with the following hypervariable regions: *HVR1-10*, *HVR2-8*, *HVR3-10*, *HVR4-8*. The specimen L16 of *L. sinapis* had *wsp*-687 allele, which differed from *wsp*-10 with one non-synonymous nucleotide substitution A193G (serine to glycine). This allele had not been previously recorded and was designated at <http://pubmlst.org> as *wsp*-687. *L. morsei* had a *Wolbachia* strain with another new allele, designated as *wsp*-688. This allele differed from *wsp*-10 with 81 nucleotide substitutions (uncorrected *p*-distance 0.169) and gaps, resulting in 41 amino acid differences, and had the following hypervariable regions: *HVR1-2*, *HVR2-267*, *HVR3-2*, *HVR4-23*.

Concordance of mitochondrial and nuclear markers in *Leptidea sinapis* vs *L. juvernica*

The CAPS approach (see 'Materials and methods') allowed us to test 36 more specimens of *L. sinapis*/*L. juvernica* in addition to those 10 in which *COI* was sequenced.

We distinguished *s*- versus *j*-alleles of the mitochondrial marker *COI* and nuclear markers *CAD* and *ITS2* in the same set of specimens. The three sets of CAPS data, for all three markers, were fully concordant: each specimen possessed either only *s*- or only *j*-alleles for all three markers. This gave us a reason to consider and further refer these specimens as belonging to the true biological species *L. sinapis* and *L. juvernica*, respectively.

The 747 bp long coding sequence of the *H1* gene of histone H1 was sequenced in 9 specimens: L10, L12 (*L. juvernica*), L15–L17 (*L. sinapis*), L50, L58 (*L. amurensis*), L60, L67 (*L. morsei*); the sequences were submitted to ENA (for the accession numbers see Table 1). *L. sinapis* and *L. juvernica* appeared to have identical primary structure of the *H1* coding sequence. Comparison of those of *L. sinapis*, *L. juvernica*, *L. morsei* and *L. amurensis* revealed 10 polymorphic sites, seven of which reside in the region coding for the C-terminal domain. As compared to the consensus *H1* coding sequence for all the four species, the *H1* sequence of *L. morsei* has two transitions, G570A and A654G, while that of both *L. sinapis* and *L. juvernica* has two transitions, G27A and G63A, and two transversions, C456G and A648C. *H1* of *L. amurensis* has three transitions G36A, G346A, G456A, and 1 transversion C453G. The substitution G346A was in the codon first position and lead to the amino acid substitution A116T, while all other above mentioned substitutions are in the third positions and synonymous. Besides, the sequenograms of both studied specimens of *L. amurensis* showed in position 306 overlapping peaks for G (as in the consensus) and T (synonymous substitutions). In one of those specimens (L50), analogous simultaneous presence of both C and T was revealed in position 219. This could result from either heterozygosity for two alleles in homologous histone gene clusters and/or cis-heterogeneity for the repeated *H1* copies in the same cluster.

Correlation of molecular markers and morphological characters in the group *L. sinapis* + *L. juvernica*

The lengths of the following genital structures were measured: the saccus and valve in males (Tables 4 and 5) and the ductus bursae in females (Tables 4 and 6). Besides, in males, we qualitatively evaluated additional characters such as the shape of saccus (straight versus S-like curved) and some wing characters (Table 7). Females of these species did not differ in external characters.

Two classes of spring brood females of the *s*- and *j*-groups with respect to the ductus bursae length were concordant with the CAPS data. The mean ductus length was significantly ($p < .001$) inferior in the *s*-group, and the length distributions of these groups did not overlap (Tables 4 and 6). In males, the difference between groups in the mean lengths of the saccus and valve were significant as well, with $p < .001$ and $p < .01$, respectively (Table 4). At the same time, the distributions of both the saccus and valve lengths of the *s*- and *j*-groups overlapped (Fig. 5).

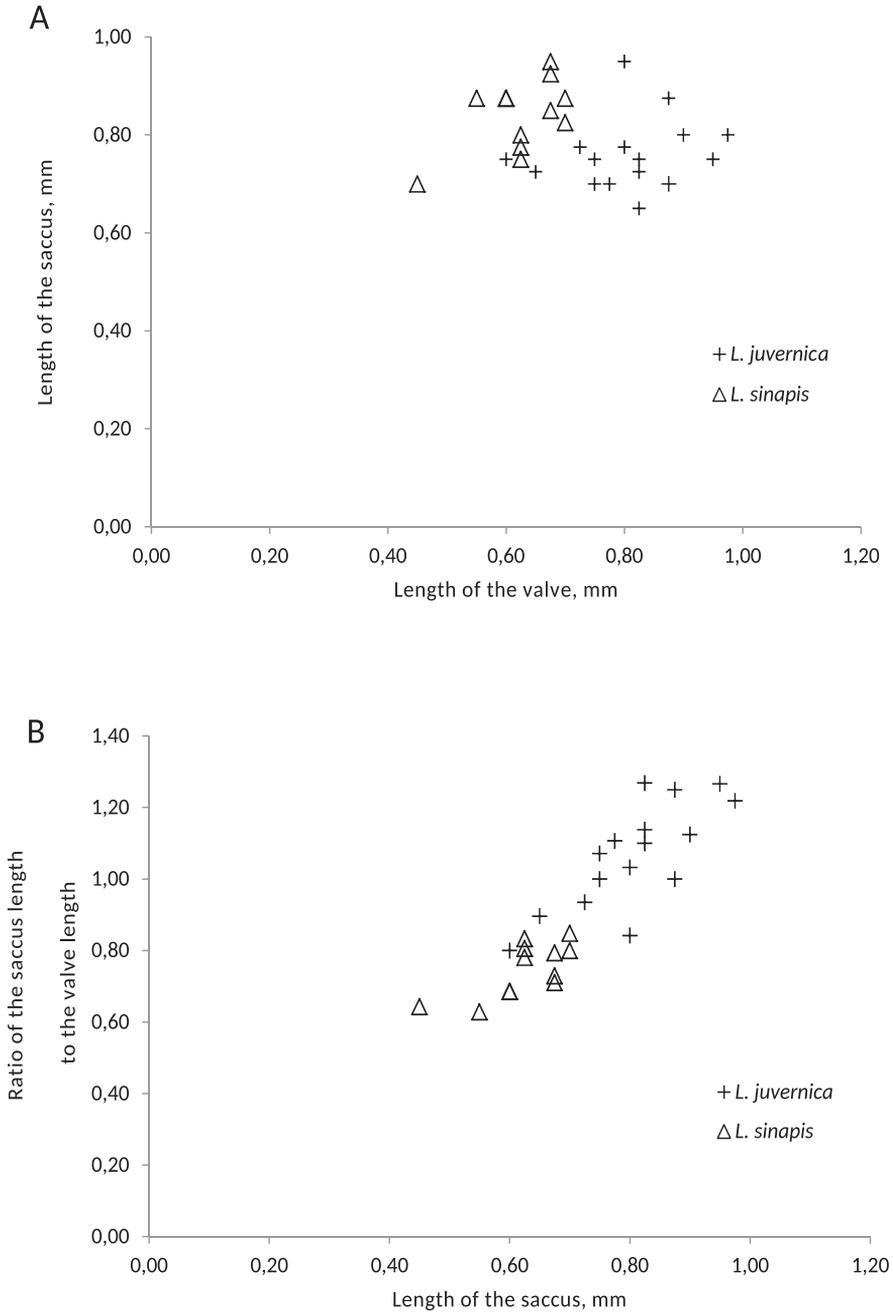


Figure 5. The saccus and valve lengths of the *L. sinapis* and *L. juvernica*. **A** Plot of the saccus length against the valve length of *L. sinapis* and *L. juvernica*, as identified by molecular markers **B** Plot of the ratio of the saccus length to the valve length against the saccus length for the same sample.

Table 4. The genital measurements of the studied samples of *Leptidea* spp. The mean values and standard deviations are given of the lengths for the saccus (*S*), valve (*V*) and their ratio (*S/V*) in the male genitalia and the length of the ductus bursae (*D*) in the female genitalia in the studied samples of *Leptidea sinapis* and *L. juvernica*, as identified by molecular markers, and the united sample of both species.

Parameter	Sample	<i>S</i> mm	<i>V</i> mm	<i>S/V</i>	<i>D</i> mm
mean	<i>L. juvernica</i>	0.81	0.76	1.07	0.96
	<i>L. sinapis</i>	0.63	0.84	0.75	0.58
	both species	0.73	0.79	0.93	0.85
standard deviation	<i>L. juvernica</i>	0.10	0.07	0.15	0.16
	<i>L. sinapis</i>	0.07	0.07	0.07	0.03
	both species	0.13	0.08	0.20	0.22
T-criterion for differentiation between the species		5.60	2.84	7.49	9.42
significance		P < 0.001	P < 0.01	P < 0.001	P < 0.001

Table 5. CAPS-analysis data, the lengths of the saccus (*S*), valve (*V*) and their ratio (*S/V*) in males of *L. sinapis* and *L. juvernica*.

Specimen	CAPS-analysis data (gene/restriction endonuclease)			Measurements		
	<i>COII</i> <i>HpaII</i>	<i>ITS2/</i> <i>AluI</i>	<i>CAD</i> <i>HindIII</i>	<i>S</i> mm	<i>V</i> mm	ratio <i>S/V</i>
L1	s	s	s	0.60	0.88	0.69
L2	j	j	j	0.83	0.73	1.14
L3	j	j	j	0.80	0.78	1.03
L4	s	s	s	0.63	0.80	0.78
L5	j	j	j	0.78	0.70	1.11
L6	s	s	s	0.45	0.70	0.64
L7	s	s	s	0.60	0.88	0.69
L8	j	j	j	0.88	0.70	1.25
L9	j	j	j	0.75	0.70	1.07
L10	j	j	j	0.60	0.75	0.80
L11	j	j	j	0.73	0.78	0.94
L12	j	j	j	0.75	0.75	1.00
L13	j	j	j	0.83	0.75	1.10
L14	j	j	j	0.90	0.80	1.13
L15	s	s	s	0.70	0.88	0.80
L16	s	s	s	0.55	0.88	0.63
L17	s	s	s	0.68	0.95	0.71
L18	s	s	s	0.68	0.93	0.73
L19	j	j	j	0.65	0.73	0.90
L20	j	j	j	0.95	0.75	1.27
L21	j	j	j	0.83	0.65	1.27
L22	s	s	s	0.68	0.85	0.79
L23	s	s	s	0.63	0.78	0.81
L24	j	j	j	0.80	0.95	0.84
L25	s	s	s	0.70	0.83	0.85
L26	s	s	s	0.63	0.75	0.83
L27	j	j	j	0.88	0.88	1.00
L28	j	j	j	0.98	0.80	1.22

Table 6. CAPS-analysis data and the lengths of the ductus in females of *L. sinapis* and *L. juvernica*.

Specimen	CAPS-analysis data (gene/restriction endonuclease)			The length of ductus (mm)
	<i>COI</i> / <i>HpaII</i>	<i>ITS2</i> / <i>AluI</i>	<i>CAD</i> / <i>HindIII</i>	
L29	j	j	j	1.00
L30	j	j	j	1.10
L31	j	j	j	0.83
L32	j	j	j	0.78
L33	j	j	j	0.95
L34	s	s	s	0.60
L35	j	j	j	1.00
L36	j	j	j	0.95
L37	s	s	s	0.60
L38	j	j	j	0.88
L39	j	j	j	0.85
L40	j	j	j	1.23
L41	j	j	j	0.75
L42	j	j	j	1.25
L43	s	s	s	0.55
L44	s	s	s	0.55
L45	j	j	j	0.90
L46	s	s	s	0.60

The saccus curvature did not appear as a reliable differentiating character as well, since its mean square contingency coefficient (φ coefficient) value with the CAPS data was rather small ($\varphi = 0.50$, $p < .001$).

The size, coloration of the hind wing underside and the shape of apex of the fore wing were also found to associate with the molecular groups *j* and *s* but again with small values of the φ coefficient: $\varphi = 0.49$ for the size, $\varphi = 0.73$ for the hind wing coloration $p < 0.001$; $\varphi = 0.29$ for the fore wing apex $p < 0.05$.

It may be concluded that neither the genital structure lengths, nor the saccus curvature, nor the general size, nor the wing coloration allow reliable identification of males of the *s*- and *j*-groups.

Discussion

Genetic integrity of the species *L. sinapis* and *L. juvernica* in the studied location

The observed differences in the studied *COI* fragment of *L. sinapis* and *L. juvernica* are substantial. They are illustrated by the averaged and minimum *p*-distances provided in Table 3 (both values being very close to each other). This result well agrees with the earlier published data (Dincă et al. 2011, 2013, Lukhtanov et al. 2011). Full concordance of alleles of two unlinked nuclear genes and mitochondrial genes unequivocally supports the

existence of integrated “molecular species” which can be identified based on diagnostic nucleotide substitutions in either of the mentioned genes, by CAPS-analysis or direct sequencing. Since any interspecies cross would bring about discordance of these markers, that we did not detect, the gene flow between these ‘molecular species’ is either absent or very limited. Thus, our ‘molecular species’ are at the same time valid biological species according to the Mayerian species concept. They are to be identified as taxonomical species *Leptidea sinapis* and *L. juvernica*, according to the predominating morphological characters used to be considered diagnostic for species bearing these names. At the same time, we claim that these morphological characters are not satisfactory for species identification, since opposite variants of each of them are still present in each of the two species.

Insufficiency of morphological and colorational characters for identification of the species *L. sinapis* and *L. juvernica*

In some studies the task of quick and still reliable identification of species of the *L. sinapis* complex by application of a morphometric approach was achieved with a 100% efficiency (Fumi 2008, Sachanowicz 2013). In other cases, overlapping of morphometric characters was observed so that some specimens could not be unequivocally identified (Hauser 1997, Kudrna 2001, Verovnik and Glogovčan 2007). This could result from either insufficient genetic isolation of species or a greater intraspecific variation, e.g. driven by ecological factors (Bolshakov et al. 2013, Tsvetkov 2007, Fumi 2008) or from differences between the spring and summer brood (Sachanowicz 2013). Fumi (2008) also noted the potential effect of choosing poor diagnostic characters and measurement errors.

According to the discriminant criterion suggested by Fumi (2008) for females of *L. sinapis* and *L. reali*, the critical value for the ductus bursae length was 0.79 mm. According to our data, the hiatus of this character between females of *L. sinapis* and *L. juvernica* is at the interval of 0.60–0.75 mm.

The length of the saccus and valve in our case appeared insufficient for a complete discrimination of *L. sinapis* and *L. juvernica*. The ratio of these values, which allowed 98% discrimination of males of *L. sinapis* and *L. reali* (Fumi 2008), in our case also had better resolution but did not yet display a hiatus between the species (Fig. 5). Ideally, the way of discriminating should be unequivocal and not depend on geographical, ecological or seasonal circumstances. Fumi (2008) achieved 100% discrimination of males of *L. sinapis* and *L. reali* through simultaneous analysis of four genitalic characters: the lengths of the aedeagus, saccus, valve and uncus, while adding further characters to the multivariate analysis did not further contribute to resolution. However, discriminative analysis of several morphometric characters of the genitalia is laborious and hence impractical for routine identification of specimens. At present, molecular analysis involving either of our CAPS-markers is an easier means of identification a specimen than multivariate analysis of the genitalia morphology. Furthermore, the differentiation of *L. reali* and *L. juvernica* is still beyond the morphological approach and until now these species can be distinguished only by molecular markers and/or karyotype (Dincă et al. 2011, 2013).

Other external characters, such as the general size and coloration of the hind wing underside, recognised by a naked eye, are unreliable and allow only a first approach to species identification in the field (Ivonin et al. 2009), as follows from the low values of the φ coefficient for association of these characters with molecular markers. This may result from the conventional nature of the character grades or from the great variability for these characters, maybe as a remnant of introgression between species in the past. Note, however, that no males of *L. juvernica* (identified by molecular markers) were scored as '0' as to the hind wing underside suffusion (that is with well-expressed lighter spots below vein M3), the variant found in the majority of males of *L. sinapis* (Table 7). This somewhat agrees with the data by Ivonin et al (2009) who did not find external characters of *L. sinapis* among the males of *L. juvernica* identified by the genitalia.

Anyway, we conclude that molecular and karyological characters are so far the only reliable means of identification of *L. sinapis* and *L. juvernica*, and the molecular ones are much easier methodically.

Overlapping lengths of genital structures of *Leptidea sinapis* and *L. juvernica*

Divergence and fixation of alleles of genes responsible for reproductive isolation are sufficient for speciation (Wu 2001). If genitalic differences contribute to reproductive isolation, genes governing the genital structures are to occur among them quite often. Differences in the genitalia size and structure result from the realization of the ontogenetic program. Since *Leptidea sinapis* and *L. juvernica* are closely related species, they should have the same set of orthologous genes with a similar system of expression regulation in ontogenesis. A new allele in one of such 'genital' genes which once occurred in a small population can soon be fixed by gene drift, giving rise to a nascent genetically isolated species. Developmental genes often have pleiotropic effects and their mutation can bring about changes in a complex of morphological characters. In particular, the same gene may affect the length of both male and female genitalia through an effect on the size of the anlagen of genital organs in early embryogenesis in both sexes. Hence, the differences in the male and female genitalia between *L. sinapis* and *L. juvernica* may be determined by the same single major gene but also be influenced by small effects of an unknown number of other genetic and/or environmental factors. This seems to be common in *Leptidea*: thus, *Leptidea lactea* Lorkovic, 1950 was isolated from *L. morsei* because of an observed bimodal distribution of the genitalia length in united samples (Lorkovic 1950).

The overlap of distribution of the length of the male genital structures may be interpreted through presence in both species of both 'long' and 'short' alleles of the hypothetical gene responsible for differences between *L. sinapis* and *L. juvernica*, although with oppositely biased frequencies. This could result from:

- inheritance of both alleles from the common ancestor,
- introgression between species, and
- de novo mutational re-appearance of 'long' and/or 'short' alleles.

Table 7. Additional characters: the saccus curvature, general size, hind wing underside suffusion and fore wing apex shape, classified to arbitrary classes, in the studied male specimens of *Leptidea sinapis* and *L. juvernica*. Character states: saccus: 0 – straight, 1 – S-like curved; general size: 0 – large, 1 – small; hind wing underside suffusion below vein M3: 0 – with well-expressed lighter spots between veins, 1 – rather even, with very scarcely or not expressed lighter spots; fore wing apex: 0 – broadly rounded; 1 – more attenuated and acute. The typical *L. sinapis* phenotype corresponds to the character states 0000, the typical *L. juvernica* to 1111.

Specimen	Molecular identification	Saccus curvature	General size	Hind wing underside suffusion	Fore wing apex
L1	s	1	0	0	0
L2	j	1	1	1	1
L3	j	1	1	1	1
L4	s	1	0	1	1
L5	j	1	1	1	1
L6	s	0	0	0	0
L7	s	0	1	1	1
L8	j	1	1	1	0
L9	j	1	1	1	0
L10	j	1	0	1	1
L11	j	1	1	1	1
L12	j	1	1	1	0
L13	j	1	1	1	1
L14	j	1	1	1	1
L15	s	1	0	0	1
L16	s	1	1	0	1
L17	s	0	1	0	0
L18	s	0	0	0	0
L19	j	1	1	1	1
L20	j	1	1	1	1
L21	j	1	1	1	0
L22	s	1	0	0	0
L23	s	0	0	0	0
L24	j	1	1	1	1
L25	s	0	0	0	0
L26	s	1	1	1	0
L27	j	0	0	1	0
L28	j	1	0	1	0

Introgression is a common phenomenon for sympatric closely related butterfly species. According to an estimation by Mallet (2005), about 16% of 440 European butterfly species can hybridise with at least one other species in natural conditions. Most of such hybrids, especially females, suffer from lowered fertility or complete sterility in F1. However, some interspecific hybrids are still able of backcrossing with one of the parental species, which can lead to gene flow in hybrid zones (Mavárez et al. 2006, Descimon and Mallet 2009).

Specimens from Novosibirsk Province with intermediate state of diagnostic external characters or, more frequently, with discordant combination of characters of *L. sinapis* and *L. juvernica* were supposed to be interspecies hybrids or products of their backcrosses (Kosterin et al. 2007, Ivonin et al. 2009). In particular, Ivonin et al. (2009) claimed that among the spring brood males with the genitalia of *L. sinapis*, there were specimens with the external characters of *L. juvernica* (a dark, evenly suffused hind wing underside below vein M3, a processed fore wing apex). Oppositely, the males with *L. juvernica* genitalia were homogenous for these characters. Our data do not support the latter claim, for the coloration of hind wing underside and the fore wing apex shape varied strongly in males of both *L. sinapis* and *L. juvernica*.

An attempt to reveal hybridization between *L. sinapis* and *L. reali* in the Pyrenees using 16 allozyme loci was unsuccessful (Martin et al. 2003). Verovnik and Glogovčan (2007) suspected a possible hybridisation between *L. sinapis* and *L. juvernica* in Slovenia. They revealed some unusual specimens which either had the saccus of an intermediate length or had a long saccus but short aedeagus. These specimens referred to *L. sinapis* according to molecular markers. The same authors revealed two specimens morphologically corresponding to *L. juvernica* but belonging to *L. sinapis* according to the *COI* sequence. However, their RAPD analysis revealed fragments specific both to *L. sinapis* and *L. juvernica*, thus suggesting the hybrid nature of those specimens. The most recent large scale study did not detect signs of introgression among the three species of the *L. sinapis* complex (Dincă et al. 2013). On the contrary, the existence of biochemical and behavioural prezygotic barriers among them were demonstrated (Friberg et al. 2008b, Dincă et al. 2013).

Inheritance of 'long' and 'short' alleles in the common ancestor of *L. sinapis* and *L. juvernica* is also a plausible interpretation. These alleles could be involved in genetic isolation of the nascent species by forming a reproductive barrier between them, but fixation of either allele in these species may not have taken place. The initial genetal prezygotic barrier could later be strengthened by adding biochemical and behavioral barriers. They would lower significance of the primary genetal barrier and somewhat release selection for the 'long' versus 'short' alleles and vice versa, allowing their frequency to drift.

At this stage of our knowledge, the third scenario of arising 'long' and/or 'short' allele(s) cannot be excluded as well.

Variability of common markers versus conservation of histone H1 gene

In contrast to core histones, histone H1 is a very variable protein (Berdnikov et al. 1993, Happel and Doenecke 2009). For this reason its gene served well for reconstructing phylogeny of the genus *Pisum* L. (peas) both at inter- and intraspecies level (Zaytseva et al. 2012, 2015). In spite of its variability elsewhere, the *H1* gene appeared identical in *L. sinapis* and *L. juvernica*. The *H1* gene variation revealed in the four studied species of *Leptidea* turned out to be five times lower than that of *COI*, differing from our data obtained for three species of *Oreta* Walker, 1955 (*Drepanidae*) (Solovyev et al. 2015), where the substitution rate in *H1* appeared to be only twice less than that in *COI*.

Three *wsp* alleles in four *Leptidea* species

All the four studied *Leptidea* species were found to be infected with *Wolbachia*, with prevalence of infected specimens of 91.8% in *L. sinapis* + *L. juvernica* and 100% in *L. amurensis* and *L. morsei* (Table 1). We cannot exclude the possibility that all individuals in the studied populations are infected. We removed the abdomen and hence isolated DNA from somatic tissues only, while the *Wolbachia* presence can be limited to reproductive tissues (Dobson et al. 1999). The high level of infection probably indicates at functional effect of *Wolbachia* on the host, ranging from mutualism (increase of the host fitness) to a reproductive parasitism (cytoplasmic incompatibility, feminization, parthenogenesis, male-killing) (Werren 1997; Werren et al. 2008). We can exclude feminisation, male-killing or parthenogenesis that would result in biased sex ratio, which was not observed (Table 1). Special experimental studies would be necessary to investigate the reason of the high infection level in these four *Leptidea* species.

Wolbachia infection is vertically transmitted through host generations via maternal cytoplasm. Therefore the phylogeny of *Wolbachia* could be expected to be concordant with the phylogeny of its hosts. However, *Wolbachia* can as well be transmitted horizontally between related species through introgression and between unrelated species by unknown agents. In addition, *Wolbachia* strains as well as their particular genes such as *wsp* may result from recombination between different strains.

We found three *Wolbachia* strains in *Leptidea* according to the *wsp* gene sequences. Three species, *L. amurensis*, *L. sinapis* and *L. juvernica*, were found to possess allele *wsp*-10 which is widespread in insects. According to our counts at the <http://pubmlst.org>, it was so far registered in 27 species of Lepidoptera from different families (Pyrilidae, Hesperidae, Papilionidae, Pieridae, Nymphalidae, Lycaenidae) and, and also in *Culex pipiens* Linnaeus, 1758 from Diptera. The second allele *wsp*-687 was found in *L. sinapis* for the first time. It differs from *wsp*-10 in only one nucleotide substitution. The third *wsp*-688 allele was also found for the first time, in *L. morsei*. This new allele has a unique hypervariable region 2, *HVR2*-267, while other hypervariable regions *HVR1*-2, *HVR3*-2, *HVR*-23 were found elsewhere (Baldo et al. 2005, 2010; Baldo and Werren 2007). Nevertheless the mentioned combination of the hypervariable regions has not been so far recorded, thus the allele is a product of recombination of strains representing different evolutionary lines of *Wolbachia*.

The pattern of *Wolbachia* variants in the studied *Leptidea* species is discordant to the host phylogeny. Variation of *wsp* sequences in *L. sinapis*, *L. juvernica* and *L. amurensis* is extremely low, viz. *wsp*-10 allele is common for these species and a closely related *wsp*-687 allele is also found in *L. sinapis*, whereas *L. morsei* possesses a highly divergent *wsp*-688 allele.

The *wsp*-10 allele could hardly be inherited from the common ancestor of the three species, taking into account a considerable degree of variation accumulated by the host *Leptidea* genes, both nuclear and mitochondrial (Dincă et al. 2011, 2013 and this paper). Note that supposition (i) contradicts also the phylogenetic relationships of the species involved (Dincă et al. 2011) as follows: the branch *L. morsei* + *L. amurensis*

is opposed to the branch containing *L. sinapis* and *L. juvernica*. The low *wsp* variation in three species may have two explanations:

(i) the same strain of *Wolbachia* could have spread across the three species by interspecies crosses;

(ii) The same *wsp* allele could have spread across the three species via horizontal transfer of *Wolbachia*.

We exclude option (i) since we failed to trace such crosses by other molecular means. Explanation (ii), that is independent infection by the same *Wolbachia* strain, is most probable because of a high frequency of *wsp-10* in butterflies. *L. morsei* was no doubt independently infected by an unusual *Wolbachia* strain with *wsp-688*, however, more data on *L. morsei* is necessary to consider the evolutionary history of its *Wolbachia*.

Conclusions

Leptidea amurensis, *L. morsei*, *L. sinapis* and *L. juvernica* coexist in the same locality in West Siberia without detectable introgression. Each of the molecular characters *COI*, *CAD* and *ITS2* markers, as well as the length of the female ductus bursae, allow a reliable identification of *L. sinapis* and *L. juvernica*. The length of the saccus related to that of the valva as the most easily assessed male genitalic character, as well as the characters of wing pattern and shape in males, are unreliable for identification of these two species. An overwhelming majority of *Leptidea* individuals are infected with *Wolbachia*. Three alleles of the *Wolbachia* gene *wsp* were recorded (two of them for the first time), that of *L. morsei* being highly divergent from the allele found in *L. amurensis*, *L. juvernica* and *L. sinapis* (this species contains a very similar third allele), which is discordant with the presumed phylogeny of the host.

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First description of B chromosomes in the *Hyphessobrycon* (Characiformes, Characidae) genus: a hypothesis for the extra element of *Hyphessobrycon eques* Steindachner, 1882

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Abstract

The *Hyphessobrycon* are allocated in the *incertae sedis* group of the Characidae family, one of the genera with more species of the group. The chromosomes of some species of *Hyphessobrycon* are known, and the diploid number most common for genus is $2n = 50$ chromosomes. The aims of this study were to examine the karyotype macrostructure in the *Hyphessobrycon eques* Steindachner, 1882, and show a new origin hypothesis for B chromosomes. The diploid number observed for *H. eques* was $2n = 52$ chromosomes, and a karyotype formulae of $12m + 18sm + 8st + 14a$, with FN (fundamental number) = 90 for both sexes. Only two females showed one B chromosome. The heterochromatin was observed mainly on centromeric regions, and in the long arm of the B chromosome. In this paper, the relationship of the B chromosome of *H. eques* with an occasional chromosome rearrangement was discussed.

Keywords

Karyotype, supernumerary chromosomes, C-banding, heteromorphism, chromosome evolution

Introduction

The *Hyphessobrycon* are allocated in the *incertae sedis* group of the Characidae family (Lima et al. 2003) with more than 130 species (e.g., Lima and Moreira 2003, Carvalho and Bertaco 2006). Among these, a species known as “Mato Grosso” has been considered *Hyphessobrycon callistus* (Boulenger, 1900) for a long time, however with the revision of Weitzman and Palmer (1997), it started to be classified as *Hyphessobrycon eques*.

The chromosomal data of the *Hyphessobrycon* genus are restricted primarily to the knowledge of the diploid number. Literature data showed that the diploid number vary between $2n = 42$ and 52 chromosomes, being $2n = 50$ chromosomes the most frequently observed number for the genus, i.e. *Hyphessobrycon scholzei* Ahl, 1937 (Arefjev 1990), *Hyphessobrycon reticulatus* Ellis, 1911 (Wlasiuk and Garcia 1996, Carvalho et al. 2002a), *Hyphessobrycon bifasciatus* Ellis, 1911 (Miyazawa 1997), *Hyphessobrycon* aff. *santae* Eigenmann, 1907 (Miyazawa 1997) and *Hyphessobrycon anisitsi* Eigenmann, 1907 (Centofante et al. 2003). According to Carvalho et al. (2002a) many species of the genus have a known chromosome set, though for many species only the haploid number has been described.

The B chromosomes have been described in many neotropical fish groups (see, for example, Maistro et al. 1992, Oliveira et al. 1997, Maistro et al. 2000, Torres-Mariano and Morelli 2008, Ferreira-Neto et al. 2012, Hashimoto et al. 2012, Silva et al. 2014). The occurrence of this type of chromosome among individuals of a population can be sporadic or commonly found for many individuals, and high frequency can be shown between them. It is also possible to find variations regarding to morphology, size, number and pattern of heterochromatin in the B chromosomes (Maistro et al. 1992, Venere et al. 1999, Cavallaro et al. 2000, Fernandes and Martins-Santos 2005, Artoni et al. 2006, Hashimoto et al. 2012, Barbosa et al. 2015).

Whereas the diversity of events described in an attempt to explain the origin and function of B chromosomes, the present study aims to demonstrate the probable origin of B chromosome in *Hyphessobrycon eques* through the study of heterochromatin, and describe for the first time the presence of an extra element in the *Hyphessobrycon* genus.

Material and methods

The *H. eques* (seven males and four females) specimens were obtained from Ribeirão Claro river (22°21'36"S, 47°30'42"W) in the state of São Paulo (SP), Brazil. The individuals were anesthetized with benzocaine (5%) and then used for cytogenetic analysis. The individuals were fixed in formaldehyde 10% and then in ethanol 70%, and placed in the ichthyological collection from Departamento de Biologia do Instituto de Biociências da UNESP, campus de Rio Claro. The chromosomes were obtained as described by Foresti et al. (1981). Chromosome morphologies were determined according to the ratio of the arms (the most frequently used classification system for

fish chromosomes). Briefly, the length of the long arm (q) was divided by the length of the short arm (p) as cited by Piscor et al. (2013). Therefore, the chromosomes with two arms and an arm ratio (AR) of 1–1.7 were classified as metacentric (m), those with two arms and AR of 1.71–3 were classified as submetacentric (sm), and those with two arms and AR of 3.01–7 were classified as subtelocentric (st). Chromosomes with a single arm (AR >7) were considered to be acrocentric (a). Heterochromatin was observed using the C-band technique proposed by Sumner (1972).

Results

The *H. eques* specimens had 2n = 52 chromosomes, and the karyotype contained 12 metacentric, 18 submetacentric, 8 subtelocentric, and 14 acrocentric chromosomes (12m + 18sm + 8st + 14a), yielding a FN of 90 for both sexes (Figure 1a, Table 1). A region of secondary constriction was evident on the short arm of one homolog of pair 19 (Figure 1a, b). One subtelocentric B chromosome was observed in all cells of two females (Figures 1b, 2a). Heterochromatic regions were observed mainly on centromeric regions, and a large block was observed in the short arm on one homolog of pair 19 (individuals with and without B chromosome) (Figure 2b). The B chromosome showed the long arm fully heterochromatic (Figure 2b).

A summary diagram indicating a possible origin mechanism of the B chromosome in *H. eques* by heterochromatin blocks is shown in Figure 3.

Table 1. Cytogenetic data and presence of B chromosomes in the *Hyphessobrycon* genus.

Species	2n	Karyotype formulae	Presence of Bs	References
<i>H. minor</i>	52	14m+20sm+16st	–	Arefjev (1989)
<i>H. scholzei</i>	50	8m+20sm+8st+14a	–	Arefjev (1990)
<i>H. flammeus</i>	52	18m/sm+32st+2a	–	Arefjev (1990)
<i>H. herbertaxelrodi</i>	52	10m/sm+42st/a	–	Arefjev (1990)
<i>H. reticulatus</i>	50	20m+14sm+16st/a	–	Wlasiuk and Garcia (1996)
<i>H. bifasciatus</i>	50	16m+10sm+12st+12a	–	Miyazawa (1997)
<i>H. aff. santae</i>	50	12m+10sm+10st+18a	–	Miyazawa (1997)
<i>H. reticulatus</i>	50	14m+20sm+16st	–	Carvalho et al. (2002a)
<i>H. reticulatus</i>	50	-	–	Carvalho et al. (2002b)
<i>H. griemi</i>	48	-	–	Carvalho et al. (2002b)
<i>H. anisitsi</i>	50	6m+16sm+12st+16a	–	Centofante et al. (2003)
<i>H. anisitsi</i>	50	18m+10sm+6st+16a	–	Mendes et al. (2011)
<i>H. luetkenii</i>	50	6m+8sm+36a	–	Mendes et al. (2011)
<i>H. eques</i>	52	14m+16sm+4st+18a	–	Martinez et al. (2012)
<i>H. eques</i>	52	12m+18sm+8st+14a	0–1 ♀/0 ♂	Present study

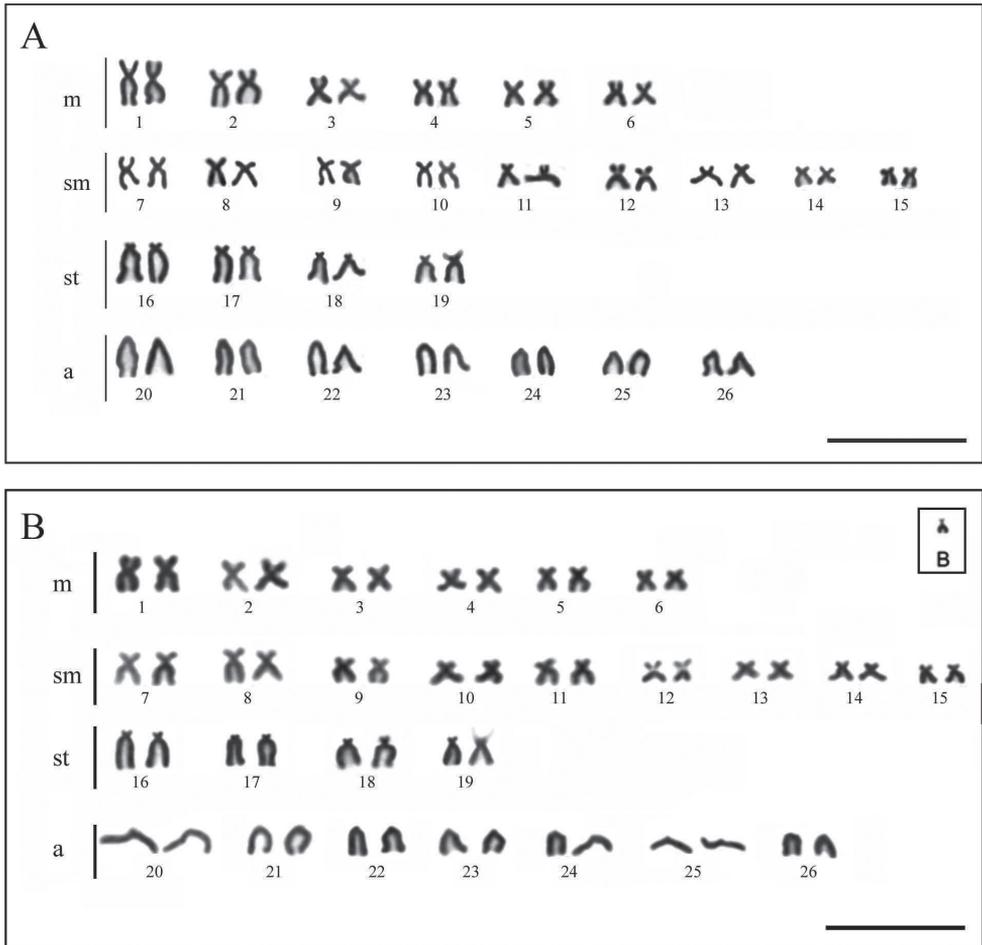


Figure 1. Giemsa stained chromosomes of *H. eques*. **A** Karyotype without B chromosome **B** Karyotype with B chromosome. Inset show the B chromosome. Bar = 10 μm.

Discussion

The heterochromatin was observed mainly in the centromeric regions on chromosomes of *H. eques* in this present paper. On the other hand, Carvalho et al. (2002a) detected small heterochromatin blocks in the pericentromeric regions in all chromosomes of *H. reticulatus* from Juquiá river (state of São Paulo, Brazil). Centofante et al. (2003) studied two populations of *H. anisitsi* from adjacent hydrographic basins (upper Paraná river basin and Paraíba do Sul river basin) and also observed heterochromatic blocks mainly on pericentromeric regions of most chromosomes.

An interesting feature observed by C-band technique in the *H. eques* was a heteromorphous block of heterochromatin always presents on short arm (pair 19) in all specimens (with and without B chromosomes), which another population of *H. eques* studied by Martinez et al. (2012) not showed. Nevertheless, we believe that the B

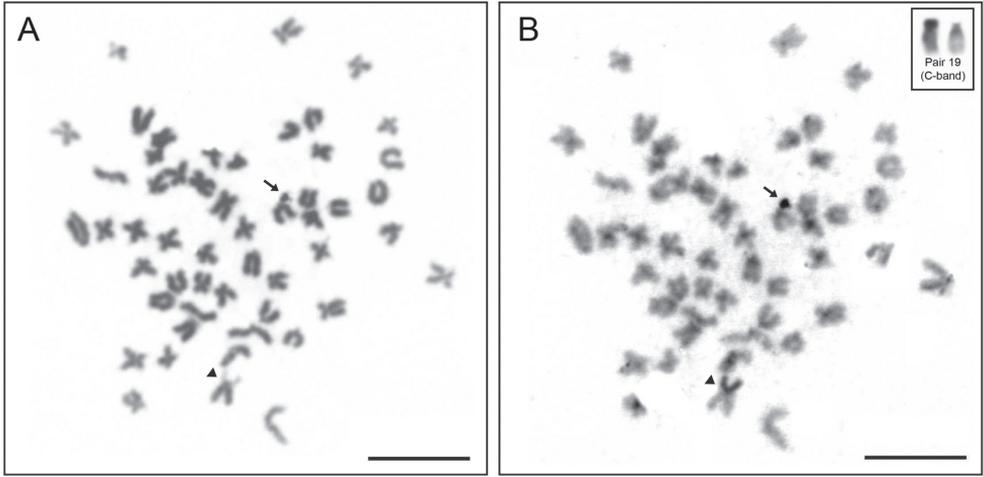


Figure 2. Mitotic metaphase chromosomes. **A** Giemsa stained **B** C-banding. The arrow indicates the B chromosome and the arrowhead indicates the secondary constriction. Inset show the pair 19 C-banded of an individual without B chromosome. Bar = 10 μ m.

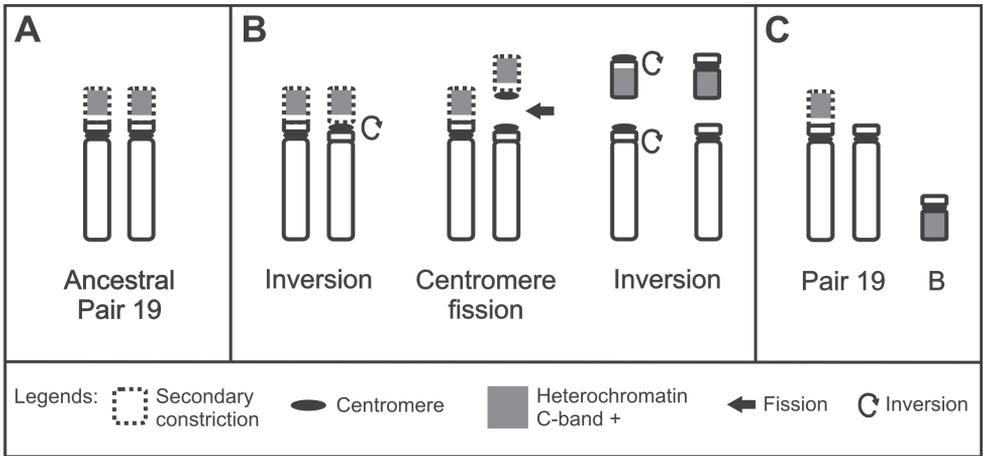


Figure 3. Scheme showing a possible origin of the B chromosome in *H. eques*. **A** Pair 19 not fissioned **B** The short arm of one homologous underwent fission and inversion **C** One homologous of pair 19 without the secondary constriction and a B chromosome formed.

chromosome (observed in two *H. eques* females) may be related with chromosomal rearrangements (see a possible mechanism in the Figure 3).

This study reported for the first time the presence of B chromosomes in the *Hyphessobrycon* genus. According to Leach et al. (2004) analyses of the molecular structure have shown that B chromosomes are subject to gene silencing, repetitive DNA accumulation and heterochromatinization. Thus, most of the heterochromatic of B chromosomes are due to the presence of chromatin characterized by a high degree of condensation during the cell cycle, and this natural condensation results from the high

content of the repetitive DNA of many types, especially satellite and ribosomal DNAs (Camacho 2005).

Different postulations have been formulated to explain the independent evolution of B chromosomes in the genome of organisms that possess them. Camacho et al. (2000) claim that, subsequent to synaptic isolation of the B chromosome and regardless of their origin, processes of molecular evolution also can occur and determine a degenerate morphology for these genomic segments. Thus, the morphological and structural features would be more a reflex of molecular evolution processes than the way in which they originated. However, it appears that the supernumerary chromosomes do not present a model of common origin, i.e. they may have originated independently following different evolutionary paths.

One hypothesis proposed to explain the presence and function of the B chromosomes is the isochromosome (Vicente et al. 1996, Mestriner 2000, Silva et al. 2014). According to Sumner (2003), isochromosomes are chromosomes with two homologous arms, i.e. which are structurally and genetically equal and may be originated by different ways. The author explains that one of the hypotheses suggested for the emergence of this type of chromosome is the fusion between two identical acrocentric chromosomes, which most likely did not occur with the B chromosome in the *H. eques* studied in this paper.

Nevertheless, the presence of one B chromosome in females may be less likely due to the sex chromosome system in the *H. eques* (even if only one sex) than involved with possible chromosomal break. However, we cannot rule out the possibility that this occasional chromosome break, from now on, may have resulted in the maintenance of this element in the females and drive to differentiation of a sex chromosome system for *H. eques*.

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General trends of chromosomal evolution in Aphidococca (Insecta, Homoptera, Aphidinea + Coccinea)

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Abstract

Parallel trends of chromosomal evolution in Aphidococca are discussed, based on the catalogue of chromosomal numbers and genetic systems of scale insects by Gavrilov (2007) and the new catalogue for aphids provided in the present paper. To date chromosome numbers have been reported for 482 species of scale insects and for 1039 species of aphids, thus respectively comprising about 6% and 24% of the total number of species. Such characters as low modal numbers of chromosomes, heterochromatinization of part of chromosomes, production of only two sperm instead of four from each primary spermatocyte, physiological sex determination, "larval" meiosis, wide distribution of parthenogenesis and chromosomal races are considered as a result of homologous parallel changes of the initial genotype of Aphidococca ancestors. From a cytogenetic point of view, these characters separate Aphidococca from all other groups of Paraneoptera insects and in this sense can be considered as additional taxonomic characters. In contrast to available paleontological data the authors doubt that Coccinea with their very diverse (and partly primitive) genetic systems may have originated later than Aphidinea with their very specialised and unified genetic system.

Keywords

Aphids, scale insects, chromosome numbers, genetic systems, evolution, phylogeny

Introduction

The name Aphidococca was recently introduced by Kluge (2010) for the taxon combining two closely related groups of Homoptera insects, aphids and scale insects. According to the paleontological data (see for example, Shcherbakov and Popov 2002, Shcherbakov 2007) scale insects (Coccinea) could originate from ancient aphids (Aphidinea) or aphid-like ancestors in the Triassic (Fig. 1). The close relationship of both groups is well supported by numerous morphological, anatomical, embryological, cytogenetic, physiological and other characters and, as it seems, is not disputed by any modern taxonomists. In the framework of cladistic taxonomy, aphids and scale insects are considered as sister groups (see for example, Wojciechowski 1992, Gullan and Cook 2007 and others) originating from a common ancestor. However, various theoretical generalizations and attempts at analysis of any biological characters of aphids and scale insects are usually done separately for these groups. Below we shall try to analyze aphids and scale insects as a united group which can be exactly contrasted to other related groups of Paraneoptera insects with particular regard to their cytogenetics.

At present, about 5000 species of aphids and 8000 species of scale insects have been recorded from all over the world (Favret and Eades in on-line "Aphid species file" database: <http://aphid.speciesfile.org>, Ben-Dov et al. in on-line "ScaleNet" database: <http://www.sel.barc.usda.gov/scalenet/scalenet.htm>). There is no general agreement on the higher classification within both groups; the number of accepted families and their relationships are disputed in the papers of different modern authors. In general, the opposite tendencies (splitting vs. integration) of the families take place in scale insect and aphid modern systematics. Thus, some modern coccidologists (for example, Hodgson 2014) accept till 33 extant families of scale insects in contrast to the 15–19 "large" traditionally accepted families (Danzig 1986, Danzig and Gavrilov-Zimin 2014), whereas the last taxonomic catalogue of aphids (Remaudière and Remaudière 1997) places all recent "true aphids" in the single family Aphididae, in contrast to the acceptance of 6–13 true aphid families by some other authors in addition to two families of "not true aphids", Adelgidae and Phylloxeridae (Börner 1952, Shaposhnikov 1964, Heie 1987, Heie and Wegierek 2009a, b). These opposite tendencies in the systematics of scale insects and aphids reflect, to our mind, the generally higher biological diversity of scale insects, which demonstrate more patterns of morphological, cytogenetic, physiological, and ecological specialization than aphids. Here, for further discussions we shall follow the system and nomenclature of Paraneoptera accepted recently in Gavrilov-Zimin and Danzig (2012) and Danzig and Gavrilov-Zimin (2014):

Phylogenetic line **Paraneoptera Martynov, 1923** (including 7 orders: Zoraptera, Copeognatha, Parasita, Thysanoptera, Homoptera, Coleorrhyncha, Heteroptera)
Cohort **Hemiptera Linnaeus, 1758** (= Condylgnatha Börner, 1904, non Hemiptera auct.)
Superorder **Thysanoptera Haliday, 1836**
Superorder **Arthroidignatha Spinola, 1850** (= Hemiptera auct. non Linnaeus, 1758;
= Rhynchota auct. non Burmeister, 1835)

Order **Coleorrhyncha Meyers & China, 1929**Order **Heteroptera Latreille, 1810** (= Hemiptera auct. non Linnaeus, 1758)Order **Homoptera auct. non Latreille, 1810**Suborder **Cicadinea Batsch, 1789**Suborder **Psyllinea Latreille, 1807**Suborder **Aleyrodinea Newman, 1834**Suborder **Aphidinea Latreille, 1802**Superfamily **Adelgoidea Annand, 1928**Superfamily **Phylloxeroidea Herrich-Schaeffer in Koch, 1854**Superfamily **Aphidoidea Latreille, 1802**Suborder **Coccoinea Fallén, 1814** (= Coccoidea auct., Gallinsecta De Geer, 1776)Superfamily **Orthezioidea Amyot & Serville, 1843** (=Paleococcoidea Borchsenius, 1950; = Archeococcidea Bodenheimer, 1952)Superfamily **Coccoidea Fallén, 1814** (=Neococcoidea Borchsenius, 1950; = Neococcidea Bodenheimer, 1952)

Within the scale insects we recognize 19 extant families (Table 1). Within the aphids we follow the system of Shaposhnikov (1964, 1985) with minor changes (taking into account some conclusions of Heie and Wegierek 2009a, b) (see Table 3), and accept 15 recent families.

In the present paper we shall try to summarize data on chromosomal numbers, karyotypes and genetic systems of Aphidococca, mainly with regard to the evolutionary significance of these data, and try to demonstrate some previously neglected parallel tendencies in the chromosomal evolution of aphids and scale insects. Two catalogues of chromosomal numbers and genetic systems are used as the basis for this discussion – the catalogue recently published by the first author (Gavrilov 2007) for scale insects, and a catalogue for

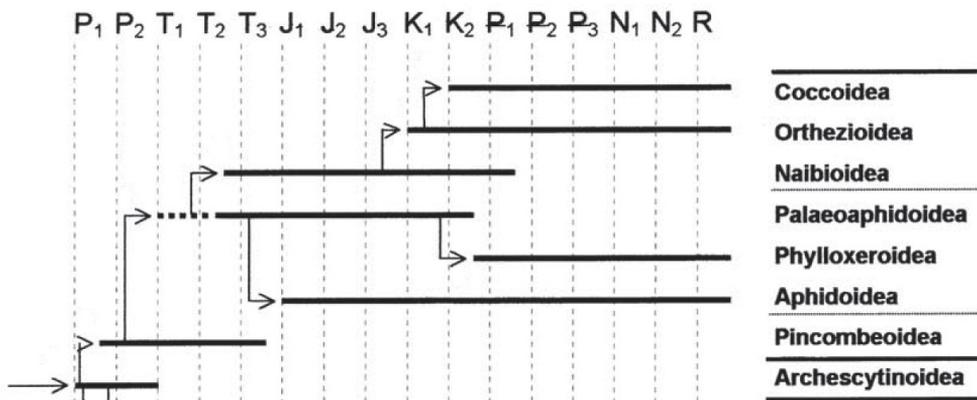


Figure 1. Phylogeny of Aphidococca placed on geochronological scale (after Shcherbakov and Popov 2002). Time periods: **P₁, P₂** Early (Lower) and Late (Upper) Permian **T₁, T₂, T₃** Early, Middle and Late Triassic **J₁, J₂, J₃** Early, Middle and Late Jurassic **K₁, K₂** Early and Late Cretaceous **P₁** Palaeocene **P₂** Eocene **P₃** Oligocene **N₁** Miocene **N₂** Pliocene **R** present time (Holocene).

Table 1. Variation of diploid chromosome number in 19 families of scale insects. **Kv** (index of karyotypic variability is provided only for the most studied families).

Family	Number of nominal taxa		Number of studied taxa		Range of variability	Kv	Modal chromosome numbers
	Genera	Species	Genera	Species			
Ortheziidae	22	202	3	3	14–18	-	-
Carayonemidae	4	4	-	-	-	-	-
Margarodidae s.l.	77	442	20	33	4–40	0.21	4, 6
Xenococcidae	3	33	-	-	-	-	-
Phenacoleachiidae	1	1	1	1	8	-	-
Pseudococcidae	279	2281	47	129	8–64	0.08	10
Eriococcidae	91	657	18	96	4–192	0.41	18
Kermesidae	10	90	1	2	26	-	-
Dactylopiidae	1	10	1	7	10–16	0.28	10
Asterolecaniidae	39	393	4	4	6–24	-	-
Stictococcidae	3	17	-	-	-	-	-
Micrococcidae	2	8	-	-	-	-	-
Acleridae	5	58	1	3	16–18	-	-
Coccidae	171	1133	27	50	10–36	0.22	16, 18
Kerriidae	9	102	2	4	18–20	-	-
Beesoniidae	6	16	-	-	-	-	-
Conchaspidae	4	30	1	1	12	-	-
Phoenicococcidae s.l. (including Halimococcidae)	6	22	5	7	10–18	-	10
Diaspididae	405	2479	68	141	6–18	0.04	8
Total	1138	7978	199	482	4–192		8, 10, 18

aphids, compiled in the present paper from numerous scattered publications on aphid cytogenetics, the main sources being the tables in Kuznetsova and Shaposhnikov (1973), Blackman (1980, 1986) and the data from the monographs of Blackman and Eastop (1994, 2006) as well as from the on-line compilation of these monographs (Blackman and Eastop 2015: <http://www.aphidsonworldsplants.info>). We hope the combined catalogue will be useful for all colleagues irrespective of any of our theoretical speculations.

Chromosome numbers

To date chromosome numbers have been reported for 482 species of scale insects belonging to 14 of the 19 known families and for 1039 species of aphids belonging to 14 families (all of those accepted here for the recent aphid fauna) (Tables 1–4), thus respectively comprising about 6% and 24% of the total number of coccid and aphid species. Thus, the greater knowledge of aphid karyotype diversity in contrast to that of scale insects is obvious at the species level as well as for the higher taxa in both these groups.

Table 2. Additions to the Gavrilov's (2007) catalogue of chromosome numbers and genetic systems of scale insects. (**H** – heterochromatinization of one haploid set of chromosomes without details of genetic system; **P(o)** – obligatory pathenogenesis).

Taxon	2n	Genetic system	Reference
Fam. Pseudococcidae			
<i>Balanococcus boratynskii</i> Williams, 1962	10	Lecanoid	Gavrilov and Trapeznikova 2010 [Belgorod Prov., Russia]
<i>Brevennia operta</i> (Borchsenius, 1949)	10	?	Gavrilov-Zimin 2011 [Turkey]
<i>Peliococcopsis priesneri</i> (Laing, 1936)	10	Lecanoid	Gavrilov-Zimin 2011 [Turkey]
<i>Phenacoccus hordei</i> (Lindeman, 1886)	10	Lecanoid	Gavrilov and Trapeznikova 2010 [Belgorod Prov., Russia]
<i>Ph. specificus</i> Matesova, 1960	10	?	Gavrilov-Zimin 2011 [Turkey]
<i>Ph. peruvianus</i> Granada de Willink, 2007	10	Lecanoid	Gavrilov and Trapeznikova 2010 [Portugal]
<i>Ph. phenacoccoides</i> (Kiritshenko, 1932)	10+B _s	Lecanoid	Gavrilov-Zimin 2011 [Turkey]
<i>Ph. prope avenae</i> Borchsenius, 1949	10	Lecanoid	Gavrilov and Trapeznikova 2010 [Portugal]
<i>Ph. tergrigorianae</i> Borchsenius, 1956	10	Lecanoid	Gavrilov-Zimin 2011 [Turkey]
<i>Puto superbus</i> (Leonardi, 1907)	16/17	XX/X0	Gavrilov-Zimin 2011 [Turkey]
<i>Rhizoecus halophilus</i> (Hardy, 1868)	10	Lecanoid	Gavrilov and Trapeznikova 2010 [Bulgaria]
<i>Trabutina crassispinosa</i> Borchsenius, 1941	16	?	Gavrilov-Zimin 2011 [Turkey]
<i>Trionymus artemisiarum</i> (Borchsenius, 1949)	10	Lecanoid	Gavrilov-Zimin 2011 [Turkey]
<i>T. haancheni</i> MckKenzie, 1960	16	Lecanoid	Gavrilov and Trapeznikova 2007 [USA]
<i>T. radicum</i> (Newstead, 1895)	10	Lecanoid	Gavrilov and Trapeznikova 2010 [Bulgaria]
Fam. Eriococcidae			
<i>Acanthococcus lactucae</i> Borchsenius, 1949	16	?Comstockioid	Gavrilov-Zimin 2011 [Turkey]
Fam. Kermesidae			
<i>Kermes roboris</i> (Fourcroy, 1785)	26	?Comstockioid	Gavrilov-Zimin 2011 [Turkey]
Fam. Acleridae			
<i>Aclerda pseudozoyisiae</i> Gavrilov-Zimin, 2012	16	H	Gavrilov-Zimin 2012 [New Guinea, Indonesia]
<i>A. takahashii</i> Kuwana, 1932	18	P(o)	Gavrilov-Zimin 2012 [Sulawesi, Indonesia]
Fam. Coccidae			
<i>Phyllostroma myrtilli</i> (Kaltenbach, 1874)	16	P, deuterotoky	Gavrilov and Trapeznikova 2008 [Bulgaria]
<i>Lecanopsis turcica</i> (Bodenheimer, 1951)	18	H	Gavrilov-Zimin 2011 [Turkey]
<i>Acanthopulvinaria orientalis</i> (Nasonov, 1908)	18 16	H H	Gavrilov 2007 [Astrakhan, Russia] Gavrilov-Zimin 2011 [Turkey]
<i>Anapulvinaria pistaciae</i> (Bodenheimer, 1926)	16?	H	Gavrilov-Zimin 2011 [Turkey]

The smallest chromosome number is the same for aphids and for scale insects, $2n=4$, and known in species of the tribe Iceryini (Coccinea: Margarodidae), in the genus *Apiomorpha* Rübsaamen, 1894 (Coccinea: Eriococcidae) (Hughes-Schrader

Table 3. Variation of diploid chromosome number in 14 families of aphids.

Family	Number of nominal taxa		Number of studied taxa		Range of variability	Kv	Modal chromosome numbers
	Genera	Species	Genera	Species			
Adelgidae	7	69	7	18	16–24	0.22	-
Phylloxeridae	8	73	4	10	6–12	0.45	-
Eriosomatidae	53	369	28	85	6–38	0.16	10, 12, 20
Mindaridae	1	9	1	2	8–12	0.30	-
Lachnidae	18	401	11	73	6–c.60	0.20	10, 12, 14
Hormaphididae	44	221	9	26	8–c.50	-	12
Thelaxidae	4	18	3	8	8–56	0.66	-
Tamaliidae	1	6	-	-	-	-	-
Aiceonidae	1	18	1	1	18	-	-
Anoeciidae	2	30	1	7	6–12	0.50	-
Phloeomyzidae	1	1	1	1	10	-	-
Greenideidae	16	178	6	21	7–40	0.36	-
Drepanosiphidae	92	573	48	141	6–c. 48	0.09	8, 14, 18
Chaitophoridae	13	178	4	39	(4?) 6–40	-	-
Aphididae	273	3033	120	605	4–72	0.03	8, 10, 12
Total	534	5177	243	1039	4–72		

1925, 1930, 1948, 1963) and in the genus *Amphorophora* Buckton, 1876 (Aphidinea: Aphididae) (Blackman 1985). The greatest numbers are $2n=72$ (in *Amphorophora sensoriata* Mason, 1923) (Blackman 1980) and $2n\approx 192$ (in *Apiomorpha macqueeni* Froggatt, 1929 (Cook 2000)). It is interesting that, both in aphids and in scale insects, the entire range of variation of chromosome number for the suborders is found in one genus in each group – *Amphorophora* in Aphidinea and *Apiomorpha* in Coccinea.

The range of diploid number variability, $2n=4-192$, demonstrated by Aphidococca is wider than in any other group of Paraneoptera, including even such huge groups as Cicadinea and Heteroptera. Thus, for the groups of Homoptera nearest to Aphidococca the following diploid chromosome numbers have been reported: Aleyrodinea, $2n=20-26$ (Blackman and Cahill 1998, but only a few species were studied until now); Psyllinea, $2n=8-26$ (Maryńska-Nadachowska 2002); Cicadinea, $2n=8-38$ (Emeljanov and Kirillova 1989, 1991). For Heteroptera the range of variability reported is $2n=6-80$ (Ueshima 1979, Kuznetsova et al. 2011), for Thysanoptera $2n=20-106$ (Brio et al. 2010), for Parasita (Mallophaga + Anoplura) $2n=4-24$ (Golub and Nokkala 2004), and for the most ancient and primitive Paraneoptera group, Copeognatha – $2n=14-30$ (Golub and Nokkala 2009).

Modal chromosomal numbers of Aphidococca as a whole, $2n=8, 10, 12, 18$ are lower (with a small overlap) than in other Homoptera, and most other Paraneoptera groups that have been sufficiently studied to provide reliable data. Thus, comparable modal numbers are $2n=26$ for Psyllinea (Maryńska-Nadachowska 2002), $2n=18, 20, 22, 26, 30$ for Cicadinea (Emeljanov and Kirillova 1989, 1991), $2n=14, 22, 26, 28,$

34 for Heteroptera (Ueshima 1979, Kuznetsova et al. 2011), and $2n=18$ for Copeognatha (Golub and Nokkala 2004). Aleyrodinea and Thysanoptera are too poorly studied for reliable comparison, but for both these groups there are no recorded chromosome numbers lower than $2n=20$. What can be a reason for the comparatively low modal numbers of Aphidococca? It is well known that there is no direct correlation between chromosomal number and complexity of an organism. On the other hand, if we look for the most general character that Aphidococca share with another group with low chromosomal numbers, the Parasita, but not with other Paraneoptera groups, we shall see that the tendency for lower modal numbers within the Paraneoptera correlates with a tendency to larvalization of imaginal structures or neoteny, with reduction of the number of postembryonic stages to three—five in Aphidococca and Parasita, in comparison with the six developmental stages usually found in most Paraneoptera.

The karyotype diversity within Aphidococca families can be characterized by a simple index of karyotypic variability (K_v) which is equal to the quantity of different diploid chromosome numbers in the taxon, divided by the number of cytogenetically studied species in this taxon. For example, in the family Diaspididae (Coccinea) six variants ($2n= 6, 8, 10, 12, 16, 18$) of the chromosomal number are known for 141 studied species. So, for Diaspididae, K_v is equal $6/141=0.04$. Of course, K_v , based on the present available data may be changed when more species are studied, but it seems this change will not be very significant. Thus, if we calculate K_v for aphid families based on the old catalogue of Kuznetsova and Shaposhnikov (1973), we obtain values similar to those based on the present catalogue (Table 3), although the number of species studied has meanwhile increased 3–4 times. It is easy to see that K_v is smallest in the largest families of Aphidococca which include numerous poorly identified (recently diverged?) species: Aphididae (0.03), Diaspididae (0.04), Pseudococcidae (0.08). On the contrary, ancient families with a limited number of recent species show comparatively large K_v -s: Adelgidae (0.22), Phylloxeridae (0.45), Margarodidae s.l. (0.21). High K_v -s in some other families, for example, Eriococcidae (0.45) or Thelaxidae (0.66), are connected mainly with enormous variability of chromosomal number not in the family as a whole, but in one of the genera (*Apiomorpha* and *Glyphina* Koch, 1856 respectively).

In the higher (above family level) taxonomic groups of Paraneoptera the utility of K_v index is currently limited by the low percentage of studied species and by limited variation of chromosomal number itself, because there are thousands of species in these higher taxa, whereas chromosomal numbers higher than $2n=60$ are very rare and higher than $2n=192$ are unknown.

Intragenetic and intraspecific chromosomal variability

A typical Aphidococca karyotype has rod-like chromosomes whose number is more or less stable within a genus (with some notable exceptions which will be discussed below). For example, in the species-rich genus *Aphis* Linnaeus, 1758, the diploid chromosome number in majority of studied species is eight ($2n=8$) with only a few exceptions.

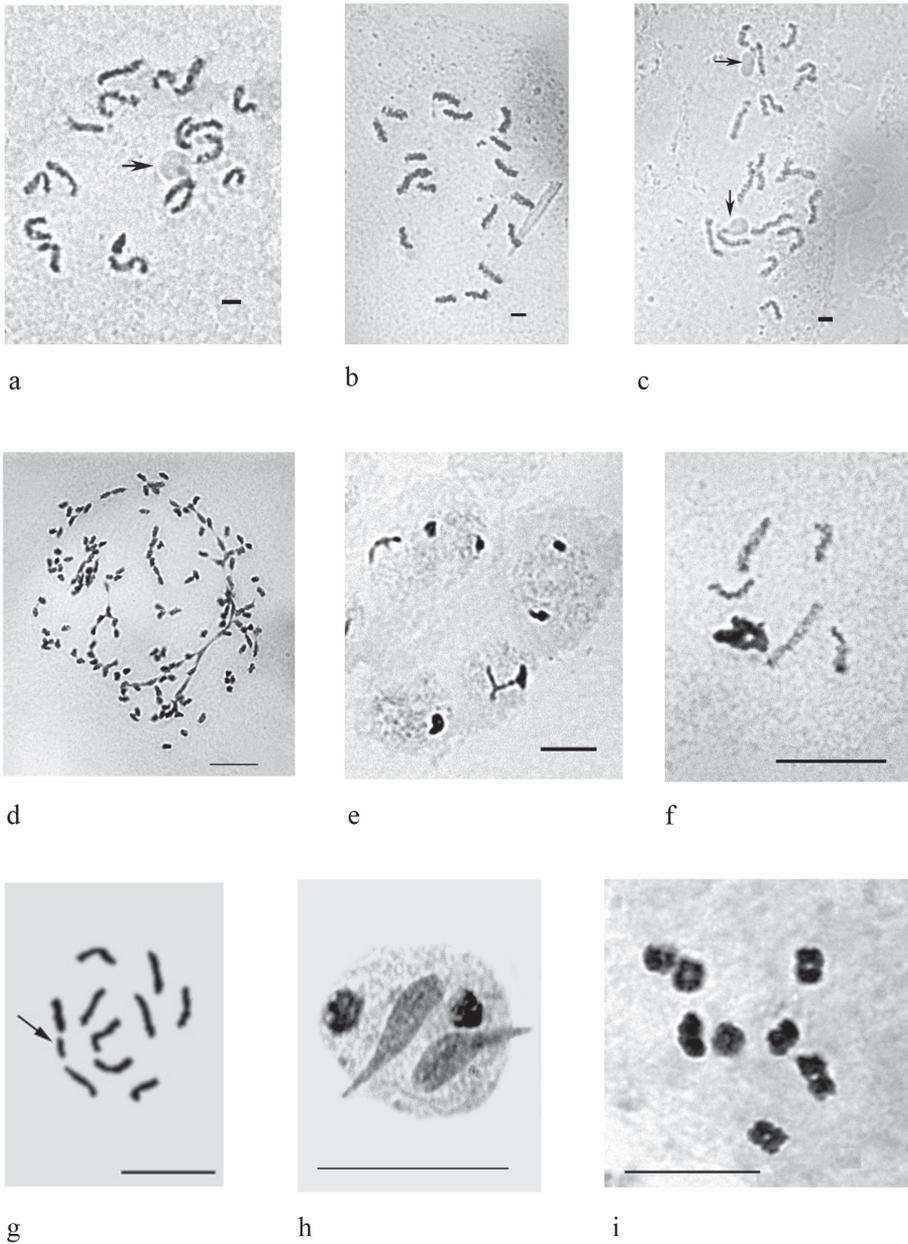


Figure 2. Mitotic and meiotic chromosomes of different scale insects. **a–c** *Puto superbus* (Leonardi, 1907), **a** cell of female embryo, $2n=18$ **b–c** cells of male embryo, $2n=17$, chromosomes with NORs are arrowed **d** *Heliococcus sulci* Goux, 1934, polyploid cell, about 140 chromosomes with numerous agglutinations **e** *Peliococcopsis priesteri* (Laing, 1936), male embryonic cells at interphase stage with one haploid set heterochromatinized **f** *Planococcus vovae* (Nasonov, 1908), male embryonic cell with one haploid set heterochromatinized **g** *Dysmicoccus multivorus* (Kiritshenko, 1936), embryonic cell with $2n=10 + B$, additional chromosomal element arrowed **h** *Chloropulvinaria aurantii* (Cockerell, 1896), $2n=26$, spermatid **i** *Protopulvinaria pyriformis* (Cockerell, 1894), $2n=16$, oogonial metaphase I. Bar = 10 μm.

Moreover, most of the species in the young and large tribe Aphidini of the family Aphididae have $2n=8$, and the same situation applies to the youngest and largest family of scale insects, Diaspididae, the overwhelming majority of species of which also have $2n=8$. On the other hand, many genera of Aphidococca demonstrate significant or even extraordinary variation of chromosome number, and, moreover, several diploid numbers can be found in the same nominal species. The most impressive example of such variation is in the scale insect genus *Apiomorpha* with its 42 diploid numbers, ranging from $2n=4$ to $2n\approx 192$ in 47 studied species (Hughes-Schrader 1925, 1930, 1948, 1963, Cook 2000, 2001). A number of aphid genera, for example, *Phylloxera* Boyer de Fonscolombe, 1834, *Glyphina* Koch, 1856, *Forda* von Heyden, 1837, *Tetraneura* Hartig, 1841, *Cinara* Curtis, 1835, *Lachnus* Burmeister, 1835, *Trama* von Heyden, 1837, *Amphorophora* Buckton, 1876, *Euceraphis* Walker, 1870, *Chaitophorus* Koch, 1854 and others also demonstrate a great variability in diploid number both between and within nominal species (see Table 4).

Polyploidy is a very rare phenomenon in Aphidococca as in other Paraneoptera and probably does not play a significant role in the evolution of the group. For scale insects a polyploid (triploid) karyotype was reported for *Physokermes hemicryphus* (Dalman, 1826) from the family Coccidae (Nur 1979), but theoretically may be found to occur in some other species of soft scales, felt scales or mealybugs which have chromosome numbers three or four times those of species known to be diploid in the same genera. In aphids polyploid species are not known at all, but several cases of polyploidization in parthenogenetic populations have been reported (see discussion in Blackman 1987). On the other hand, females usually have highly polyploid cells (Fig. 2d) in bacteriomes, peculiar organs which include intracellular symbiotic bacteria.

Accessory chromosomal elements have been found in several species of mealybugs (Pseudococcidae) (Nur et al. 1987, Gavrillov 2007) (Fig. 2g), in one species of the Margarodidae (Hughes-Schrader 1942), in two species of soft scales (Coccidae) (Gavrillov 2007) and in some armored scales (Diaspididae) (Brown 1960). Blackman (1980, 1990) noted presumed B-chromosomes in numerous aphid species from different families, especially in anholocyclic populations, and these B-chromosomes are probably relicts of multiple X-chromosomes.

Evolution of genetic systems

In contrast to other Paraneoptera, all Aphidococca have spermatocyte and oocyte meiosis in larvae or in neotenic females (which are in fact equivalent to larvae as in scale insects) and demonstrate a multiplicity of very different and unique genetic systems, which are probably based on an original XX-X0 system, considered by Blackman (1995) as ancestral for all Paraneoptera insects (Fig. 3). In species possessing this system, the sex of the progeny is determined during spermatogenesis. Spermatozoa with X-chromosomes produce females and spermatozoa without X-chromosomes produce males. This usual type of XX-X0 spermatogenesis (similar to that of Copeognatha, for example) is known

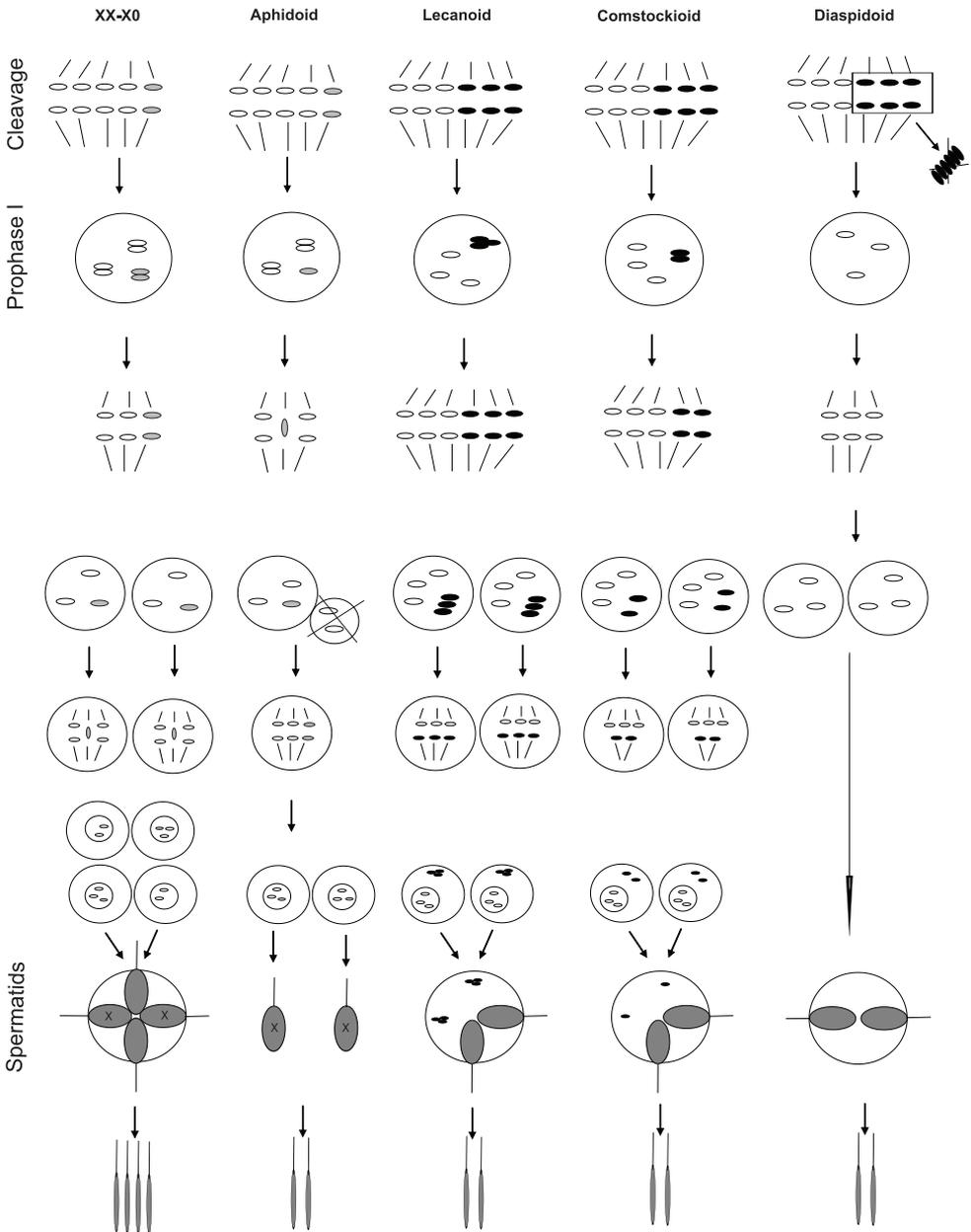


Figure 3. Meiosis and spermatogenesis encountered in different genetic systems of Aphidococca.

in some primitive scale insects (some Margarodidae, Ortheziidae, genus *Puto* Signoret, 1875 (Pseudococcidae)) (Hughes-Schrader 1931, 1942, 1944, 1955, Brown and Cleveland 1968) with only one peculiar character – spermatocytes fuse to form a quadrinucleate spermatid (Fig. 3). This fusion can be considered as a unique apomorphy of Coccinea. In some genera of Margarodidae, such as *Aspidoproctus* Newstead, 1901, *Protortonia*

Townsend, 1898, *Llaveia* Signoret, 1876, *Llaveiela* Morrison, 1927, *Nautococcus* Vaysière, 1939 (all from the subfamily Monophlebinae) XX-X0 spermatogenesis is also complicated by the enclosure of meiotic prophase I chromosomes in peculiar separate vesicles, instead of a single nuclear membrane. This phenomenon was discovered by F. Schrader and S. Hughes-Schrader and was comprehensively reviewed by Hughes-Schrader (1948). Moreover, it is interesting to note that in *Protortonia* (Coccinea: Margarodidae), in the second meiotic division, all chromosomes form a chain stretched between the two poles of the cell (Schrader 1931), which is similar to the well-known example of chain formation in plants of the genus *Oenothera* Linnaeus, 1753 (Onagraceae) and some other plants and animals (White 1973).

In most cases, species with the XX-X0 system have only one pair of sex chromosomes in their karyotypes. For example, females of *Porphyrophora polonica* (Linnaeus, 1758) (Coccinea: Margarodidae) have $2n=12+XX$ and males have $2n=12+X$. However examples of multiple sex chromosomes are also known. Thus, species of the family Adelgidae (Aphidinea) have up to four pairs of X chromosomes, and some species of the families Phylloxeridae, Eriosomatidae, Lachnidae and Drepanosiphidae (Aphidinea) have one-two pairs of sex chromosomes (see Table 4). In scale insects, only *Matsucoccus gallicolus* Morrison, 1939 (Margarodidae) has a multiple sex chromosome system with 6 pairs of X chromosomes ($2n=28+12X$ in females and $2n=28+6X$ in males), which probably evolved as a result of fragmentation of an initial pair of X chromosomes (Hughes-Schrader 1948) and it seems the number of sex chromosomes in this species is the highest known in Insecta. Multiple sex chromosomes are also known in Cicadinea and Heteroptera and can be probably considered as a non-unique apomorphic character in different genera of proboscidian insects (Arthroidignatha). This character is not known in studied Copeognatha (Golub and Nokkala 2009), which is considered as an ancestor group for proboscidians.

Hales (1989) reported a peculiar fusion of multiple X chromosomes with autosomes (X_1+A , X_1 , X_2+A , X_2) in somatic cells of *Schoutedenia lutea* (van der Goot, 1917) (Aphidinea, Greenideidae), that demonstrates a special genetic system unknown in other aphids and in Paraneoptera as a whole, but this phenomenon needs further investigation.

However, in the majority of studied scale insects and in all studied aphids sex determination is not brought about by stochastic combination of male and female chromosome sets during fertilization, because male and female gametes in most Aphidococca are cytogenetically identical and physiological sex determination takes place. Thus, in all studied Aphidinea, gametogenesis is of a unified type and based on an XX-X0 mechanism, but has unique features which are probably unknown in any other animals with XX-X0. One of the secondary spermatocytes (which includes autosomes only) is smaller in size and degenerates just after anaphase I. The second, larger spermatocyte gives origin to two sperms; both with one X-chromosome (see Manicardi et al. 2015 and our Fig. 2). Thus, aphid males give rise only to female-producing sperm, and sexual females also produce only female-producing oocytes, so that all sexually-produced progeny are female. On the other hand, parthenogenetic females can pro-

duce embryos which are either XX or X0, using a special cytological mechanism in which the X-chromosome is lost in some of the oocytes (Orlando 1974, Blackman and Hales 1986). Thus, sex of progeny is totally dependent on the physiology of the parthenogenetic female, which starts to produce sexuales under certain environmental conditions. This mode of gametogenesis is closely connected with cyclic parthenogenesis and is undoubtedly a unique apomorphy of Aphidinea. In general we suggest that the genetic system of aphids could be termed the Aphidoid system for the uniformity with the names of the genetic systems of scale insects (see below).

The majority of scale insects (almost whole superfamily Coccoidea) and aphids of the tribe Tramini (Lachnidae) demonstrate specific heterochromatinization of part of chromosomes in their diploid set. The species of scale insects with Lecanoid, Comstockioid, and Diaspidoid genetic systems feature obligate heterochromatinization of the paternal set of chromosomes in the males (Fig. 2e–f). Paternal genome heterochromatinization (PGH) is known in some groups of insects (see reviews of White 1973 and Normark 2003), but in each of these groups PGH has specific characters and forms unique genetic systems. The coccid species with systems Lecanoid, Comstockioid, and Diaspidoid can be purely sexual with identical male and female gametes, or demonstrate diploid arrhenotoky and deuterotoky in addition to heterochromatinization of the paternal set of chromosomes. In all these cases the sex of the progeny depends on rather enigmatic physiological processes occurring inside the female, as in the Aphidoid system.

In the Lecanoid system, the heterochromatic chromosome set exists during all stages of the male life cycle. During meiosis in the male, the chromosomes do not pair and separate equationally during the first division. During the second division, two metaphase plates are formed, and the heterochromatic and euchromatic chromosomes then segregate to the opposite poles (Hughes-Schrader 1948, Nur 1980). As a result of meiosis, quadrinucleate spermatids are formed, but only the nuclei of maternal origin produce sperm (Fig. 3).

In the Comstockioid system, the heterochromatic set is partly (as separate chromosomes) eliminated during embryogenesis and different cells of the same tissue may differ in chromosome number. According to the number of eliminated chromosomes, several variants of the Comstockiella system are known: CL^1 – Comstockioid-Lecanoid intermediates, C^{varH} – Comstockioid with one pair of paternal chromosomes, retained in different cysts, C^C – complete Comstockioid. The course of spermatogenesis varies among the different taxa, depending on the number of non-eliminated heterochromatic chromosomes. If all these chromosomes are destroyed, the second division is absent (Brown 1965, 1967, Nur 1980).

In the Diaspidoid system, the heterochromatic set has been completely lost, and adult males are haploid. Hence, spermatogenesis consists of a single equational division (Brown 1965, 1967, Nur 1980, 1982).

In the aphid tribe Tramini (Lachnidae), almost all studied populations reproduce by thelytokous parthenogenesis and sex chromosomes have not been identified (Blackman 1980, 1990, Blackman et al. 2000). Some of the chromosomes in the diploid

set demonstrate heterochromatinization and even aggregation of heterochromatic elements in somatic cells until late prophase (Blackman 1980), thus resembling the Lecanoid-Comstockioid genetic system in scale insects. However, heterochromatic chromosomes in Trimini can vary significantly in number between populations and do not constitute a haploid set. These heterochromatic elements of Trimini are similar to B-chromosomes, and Blackman et al. (2000) suggest that they may be derived from ancestral redundant X chromosomes.

In the tribe Fordini (Aphidinea: Eriosomatidae), germ-line and somatic cells have radically different chromosome numbers (Blackman 1980). Unfortunately this very interesting phenomenon has not been additionally studied.

Hermaphroditism and *Haplo-diploidy* are known only in species of the tribe Iceryini (Coccinea: Margarodidae) (Hughes-Schrader 1948, 1963). The hermaphrodites are diploid and similar to females in their morphology and mode of life. During embryogenesis the gonads of these insects do not undergo sexual differentiation. Later, in the crawlers, haploid nuclei appear in the gonads and form the central testicular part of a hermaphroditic gland. The haploid nuclei appear as a result of degeneration and elimination of one set of chromosomes. The peripheral ovarian part of the gland is diploid and formed a little later. Fertilization takes place either in the ovarian part or in the cavity of the ovo-testis. Fertilized eggs always develop into female-like hermaphrodites, which usually reproduce by self-fertilization. However, the hermaphrodites may also copulate with accidental haploid males, which sometimes develop from unfertilized eggs (Hughes-Schrader 1948). *Haplo-diploidy* is known in Iceryini scale insects only and is in fact a result of haploid arrhenotoky as in other insects with haploid males. Fertilized eggs produce diploid females and unfertilized eggs produce haploid males (Hughes-Schrader 1948).

To date, species with heteromorphic sex chromosomes (genetic system XX/XY, neo-XX/XY) have not been found among *Aphidococca* in contrast to larger groups of Paraneoptera: Cicadinea + Heteroptera, where these systems are very common and to Psyllinea + Copeognatha, where XX/XY (or neo-XX/XY) system is known in several species. On the other hand, in some species of scale insects, such as *Newsteadia* sp., *Praelongorthezia praelonga* (Douglas, 1891) (both from Ortheziidae), *Lachnodioides eucalypti* (Maskell, 1892) (Eriococcidae), and *Stictococcus* sp. (Stictococcidae), both females and males have the same number of chromosomes, but without distinct sex chromosomes or peculiar heterochromatinization of the paternal set (as in the unique coccid systems Lecanoid, Comstockioid, and Diaspidoid). Thus, the Australian felt scale *Lachnodioides eucalypti*, having $2n=18$ in both females and males (Brown, 1967, 1977, Nur, 1980), is especially noteworthy. In other studied species of the genus *Lachnodioides* Maskell, 1896 and in the family Eriococcidae as a whole, the Comstockioid system has been discovered, but in males of *L. eucalypti* heterochromatinization of the paternal set is absent. The $2n-2n$ system probably evolved in scale insects more than once and from different ancestral systems: from the system with heterochromatinization in *L. eucalypti* and *Stictococcus* sp. and from the XX-X0 system in *Praelongorthezia praelonga* (Nur 1980). Meiosis in *L. eucalypti* comprises one reductional division only (Brown and Chandra 1977), whereas in *P. praelonga* it comprises two divisions without an inverted meiotic sequence (Brown 1958).

Parthenogenesis

It seems that absolutely all aphid species and many scale insects can produce their progeny by parthenogenesis. In aphids the parthenogenesis can be cyclic (with alternation of thelytoky and deuterotoky – the apomorphic condition for Aphidinea) or anholocyclic (with continuous thelytoky). In scale insects no examples of cyclic parthenogenesis are known and parthenogenesis can be thelytokous, deuterotokous or arrhenotokous. On the other hand, there are probably a few obligatory thelytokous species of scale insects, such as *Protopulvinaria pyriformis* (Cockerell, 1894) and *Eupulvinaria peregrina* Borchsenius, 1953 (Gavrilov and Trapeznikova 2008), which never produce males in any population or geographical region. A great many species, often reported as thelytokous (see, for example, Nur 1990 for the review), in reality combine thelytokous reproduction with amphimixis, producing males amphimictically or parthenogenetically (diploid arrhenotoky and deuterotoky), and these males have, as usual for scale insects, paternal genome heterochromatinization. Some species variously manifest thelytokous and sexual lineages in different geographical regions or on different host plants (Nur 1990). Haploid arrhenotoky (noted above for Icerini) is connected with haplo-diploidy and can be interpreted as facultative, rather than obligatory parthenogenesis.

Unfortunately it is impossible to say now exactly how many scale insects species are able to reproduce by parthenogenesis, and this ignorance hampers a detailed comparison of scale insects and aphids in this respect.

Conclusion

Finally we can underline the following parallel trends in the evolution of Aphidinea and Coccinea:

- 1) Low modal numbers of chromosomes.
- 2) Heterochromatinization of part of chromosomes.
- 3) Production of only two sperms instead of four from each primary spermatocyte.
- 4) Physiological sex determination.
- 5) "Larval" meiosis.
- 6) Widely distributed parthenogenesis.
- 7) Intraspecific chromosomal races (some of which may be cryptic species).

We consider that at least some of these tendencies may be regarded as additional taxonomic characters, which support the erection of Aphidococca as a higher category differing radically from other Homoptera and more widely from all Paraneoptera groups.

A comparison of cytogenetic data between the two groups of Aphidococca shows that Coccinea demonstrate much more diverse cytogenetic characteristics than Aphidinea. From the cytogenetic point of view Coccinea seem to be more primitive, including specialized (in most families) as well as ancient plesiomorphic characters (in

some families): a simple XX-X0 genetic system with production of 4 sperms from one primary spermatocyte, chromosomal (not physiological) sex determination, simple bisexual reproduction, and later initiation of meiosis, i.e. characters which have been lost completely in all studied aphids. This deduction contradicts the current interpretation of paleontological data (discussed in the Introduction). It is difficult to imagine that the diverse (and partly primitive) cytogenetic mechanisms of scale insects could have originated from the very specialised and unified Aphidoid genetic system. We therefore suppose that the ancient scale insects originated at least not later than ancient aphids. The contradiction with the paleontological record may be explained by the well-known incompleteness of this record and the very limited number of taxonomic characters for fossil groups (mainly wing venation in ancient Aphidococca), which results in a very subjective identification of fossil insects. Thus, for example, fossil Naibiidae were described by Shcherbakov (1990, 2007) as most ancient, four-winged scale insects, but the same group is considered to be aphids by some aphidologists (see, for example, Wojciechowski 1992). The Lower Jurassic *Mesococcus asiaticus* Becker-Megdisova, 1960, which demonstrates the unique facies of a neotenic scale insect female, was considered by the original author as an ancient scale insect, similar to modern Monophlebinae, but was excluded from scale insects (and not placed in any other group!) by Koteja 1990.

Table 4. Chromosome numbers and genetic systems of Aphidinea. **P(c)** – cyclic parthenogenesis, **P(o)** – obligatory parthenogenesis in anholocyclic species, **B** – additional chromosomes.

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
Superfam. PHYLLOXEROIDEA				
Fam. Adelgidae				
<i>Adelges geniculatus</i> (Ratzeburg, 1844)	P(o)	20		Steffan 1968b [Germany; Canada]
<i>A. laricis</i> Vallot, 1836	P(c)	22, 21, 20/18	2(X ₁ X ₂ X ₃ X ₄)/ X ₁ X ₂ X ₃ X ₄ 0	Frolowa 1924 (as <i>Chermes strobilobius</i>) [Moscow, Russia]
		20/18	2(X ₁ X ₂)/ X ₁ X ₂ 0	Steffan 1968b [Germany]
<i>A. tardus</i> (Dreyfus, 1888)	P(o)	20		Steffan 1968b [Germany]
<i>Aphrastasia pectinatae</i> (Cholodkovsky, 1888)	P(c)	20/18	2(X ₁ X ₂)/ X ₁ X ₂ 0	Frolowa 1924 (as <i>Chermes</i>) [Moscow, Russia]
<i>Cholodkovskya viridana</i> (Cholodkovsky, 1888)	P(o)	24		Steffan 1968b [Germany]
<i>Dreyfusia nordmannianae</i> (Eckstein, 1890)	P(c)	22		Steffan 1968b [Germany]
<i>Gillettella cooleyi</i> (Gillette, 1907)	P(c)	22/20	2(X ₁ X ₂)/ X ₁ X ₂ 0	Steffan 1968a, b [Germany; Canada]
<i>G. coweni</i> (Gillette, 1907)	P(o)	22		Steffan 1968a, b [Canada]
<i>Pineus boernerii</i> Annand, 1928	P(o)	16		Blackman and Eastop 1994 [Hawaii, USA]
		17		Blackman and Eastop 1994 [California, USA; Africa; Australia; New Zealand]
<i>P. cembrae</i> (Cholodkovsky, 1888)	P(c)	18		Blackman and Eastop 1994 [?]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>P. orientalis</i> (Dreyfus, 1889)	P(c)	20		Blackman and Eastop 1994 [?]
<i>P. pineoides</i> (Cholodkovsky, 1903)	P(o)	17		Blackman and Eastop 1994 [?]
		22		Steffan 1968b [Germany; Canada]
<i>P. pini</i> (Goeze, 1778)	P(o)	19		Blackman and Eastop 1994 [New Zealand]
		20		Blackman and Eastop 1994, Blackman et al. 1995 [Europe]
		21		Blackman and Eastop 1994, Blackman et al. 1995 [Australia]
		22		Steffan 1968b [Germany]
<i>P. similis</i> (Gillette, 1907)	P(o)	22		Steffan 1968b [Canada]
<i>P. strobi</i> (Hartig, 1839)	P(o)	20		Blackman and Eastop 1994 [?]
		22		Steffan 1968b [Germany; Canada]
<i>P. (Pineoides) pinifoliae</i> (Fitch, 1858)	P(c)	22		Steffan 1968b [Canada]
<i>Sacchiphantes abietis</i> (Linnaeus, 1758)	P(o)	18		Pagliari 1967 [Italy], Steffan 1968b [Germany]
		20		Steffan 1968b (as <i>S. laricifoliae</i> (Fitch, 1858)) [Canada; USA]
<i>S. viridis</i> (Ratzeburg, 1843)	P(c)	18/16	2(X ₁ X ₂)/ X ₁ X ₂ 0	Steffan 1968a, b [Germany]
Fam. Phylloxeridae				
<i>Aphanostigma piri</i> (Cholodkovsky, 1903)	P(c)	8		Wysoki and Swirsky 1970 [Israel]
<i>Daktulosphaira vitifoliae</i> (Fitch, 1851)	P(c), P(o)	10/9	XX/X0	Maillet 1957 [France]; Forneck et al. 1999 [Europe; USA]
<i>Moritzziella caryaefoliae</i> (Fitch, 1856)	P(o)	8		Morgan 1909b (as <i>Phylloxera</i>) [USA]
<i>Phylloxera caryaecaulis</i> (Fitch, 1855)	P(c)	8/6	2(X ₁ X ₂)/ X ₁ X ₂ 0	Morgan 1909a, 1912, 1915 [USA]
<i>Ph. caryaefallax</i> Riley, 1875	?	12		Morgan 1909a, 1912, 1915 (as <i>Ph. fallax</i>) [USA]
<i>Ph. caryaeglobuli</i> Walsh, 1863	?	22		Morgan 1906, 1909b [USA]
<i>Ph. depressa</i> (Shimer, 1869)	?	6		Morgan 1909b [USA]
<i>Ph. globosa</i> (Shimer, 1867)	?	6		Morgan 1906, 1909b [USA]
<i>Ph. quercus</i> Boyer de Fonscolombe, 1834	P(c), P(o)	12/11	XX/X0	Maillet 1957 [France]
<i>Ph. subelliptica</i> (Shimer, 1869)	?	6		Morgan 1909b [USA]
<i>Phylloxera</i> sp.	?	12		Morgan 1906 [USA]
Superfam. APHIDOIDEA				
Fam. Eriosomatidae				
<i>Aloephagus myersi</i> Essig, 1950	P(c), P(o)	22		Blackman and Eastop 1984 [?]
<i>Aploneura lentisci</i> (Passerini, 1856)	P(c), P(o)	16		Blackman 1980 (as <i>Asiphum</i>) [Great Britain], Blackman and Spence 1996 [Great Britain]
<i>Appendiseta robiniae</i> (Gillette, 1907)	P(c)	10		Blackman and Eastop 1994 [?]
<i>Baizongia pistaciae</i> (Linnaeus, 1767)	P(c), P(o)	24		Blackman 1980 [Great Britain] (anholocyclic population)

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Colopha compressa</i> (Koch, 1856)	P(c), P(o)	16		Blackman 1980 [Great Britain]
<i>C. kansugei</i> (Uye, 1924)	P(o), ?P(c)	10		Blackman 1986 [Japan]
<i>Colophina arma</i> Aoki, 1977	P(c)	10 (female), 8 (male)	2(X ₁ X ₂)/ X ₁ X ₂ 0	Blackman 1986 [Japan]
<i>C. clematicola</i> (Shinji, 1922)	?P(c), P(o)	20		Blackman and Eastop 2015 [?]
<i>C. clematis</i> (Shinji, 1922)	P(c), P(o)	10 +B (female), 8+B (male)	2(X ₁ X ₂)/ X ₁ X ₂ 0	Blackman 1986 [Japan]
<i>Epipemphigus imaicus</i> (Cholodkovsky, 1912)	P(c)	18		Khuda-Bukhsh 1980, Khuda-Bukhsh and Pal 1983a [Garhwal, Uttarakhand, India]
<i>E. nisimae</i> (Matsumura, 1917)	P(c)	20		Blackman 1986 [Japan]
<i>Eriosoma crataegi</i> (Oestlund, 1887)	P(c)	12		Robinson and Chen 1969a [Canada]
<i>E. lanigerum</i> (Hausmann, 1802)	P(c), P(o)	12		Baehr 1908, 1909 (as <i>Schizoneura</i>) [Germany], Pagliai 1963 [Italy], Sun and Robinson 1966, Harper and MacDonald 1966, Robinson and Chen 1969a [Canada], Kulkarni and Kacker 1980 [India], Gautam and Verma 1982, Kulkarni 1984 [Shimla, Himachal Pradesh, India]
		12/11	XX/X0	Gautam and Verma 1983 [Shimla, Himachal Pradesh, India]
<i>E. (Mimaphidus) lanuginosum</i> (Hartig, 1839)	P(c)	10		Blackman and Eastop 1984 [?]
<i>E. (M.) patchiae</i> (Börner & Blunck, 1916)	P(c)	10		Blackman 1980 [Great Britain]
<i>E. (Schizoneura) auratum</i> Akimoto, 1983	P(c)	12		Blackman 1986 [Japan]
<i>E. (S.) grossulariae</i> (Schüle, 1887)	P(c)	10		Blackman and Eastop 1984 [?]
<i>E. (S.) harunire</i> Akimoto, 1983	P(c)	10		Blackman 1986 [Japan]
<i>E. (S.) japonicum</i> (Matsumura, 1917)	P(c)	10		Blackman 1986 [Japan]
<i>E. (S.) kashmiricum</i> Ghosh, Verma & Raychaudhuri, 1976	P(c)	12		Pal and Khuda-Bukhsh 1983 [Garhwal, Uttarakhand, India]
? <i>E. (S.) laciniatae</i> Pashtshenko, 1988	P(c)	16		Blackman and Eastop 1994 [?]
<i>E. (S.) longicornutum</i> Akimoto, 1983	P(c)	10		Blackman 1986 [Japan]
<i>E. (S.) moriokense</i> Akimoto, 1983	P(c)	10		Blackman 1986 [Japan]
<i>E. (S.) ulmi</i> (Linnaeus, 1758)	P(c)	10		Blackman and Eastop 1984 [Europe]
		12		Baehr 1908, 1909 (as <i>Schizoneura</i>) [Germany]
<i>E. near ulmi</i> (Linnaeus, 1758)	?	16		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>E. (S.) yangi</i> Takahashi, 1939	P(c)	10		Blackman 1986 [Japan]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Forda formicaria</i> von Heyden, 1837	P(c), P(o)	20		Robinson and Chen 1969a [Canada], Blackman and Spence 1996 [Great Britain]
		18-20 (somatic cells) or 21-23 (germline cells)		Blackman 1987a [Great Britain; Czechoslovakia; Sicily; Italy; Cyprus; Israel; Iran; USA; Canada]
		21, 22, 23		Blackman 1980 [Great Britain; North America] (anholocyclic populations)
<i>F. hirsuta</i> Mordvilko, 1928	P(c), P(o)	18		Blackman 1980, 1987a [Iran]
<i>F. marginata</i> Koch, 1857	P(c), P(o)	17-20 (somatic cells) or 25-40 (germ line cells)		Blackman 1987a [Great Britain; Sicily, Italy; Cyprus; Israel; Iran; USA; Canada]
		24,25, 26, 27, 32		Blackman 1980 [Great Britain; North America] (anholocyclic populations)
		28		Robinson and Chen 1969a [Canada]
<i>F. riccobonii</i> (Stefani, 1899)	P(c)	18		Khuda-Bukhsh and Pal 1983b [Gharwal, Uttarakhand, India]
		30 (germ line cells)		Blackman 1980 (as <i>F. dactylidis</i> Börner, 1950) [Iran] (but see Blackman 1987a: "Dr. V. F. Eastop has re-examined it and considers it to be closer to <i>F. riccobonii</i> (Stefani)")
		18 (somatic cells) or 30 (germ line cells)		Blackman 1987a [Iran]
<i>Formosaphis micheliae</i> Takahashi, 1925	?P(o)	10		Blackman 1986 [Japan] (with structural heterozygosity)
<i>Geoica lucifuga</i> (Zehnter, 1897)	P(c), P(o)	14		Kulkarni 1984 [Darjeeling, West Bengal, India]
		18		Blackman and Eastop 1994 [?]
<i>G. ?runsi</i> Davatchi & Remaudière, 1957	P(c)	18		Blackman and Eastop 2015 [?] (holocyclic populations on <i>Pistacia atlantica</i>)
<i>G. setulosa</i> (Passerini, 1860)	P(c), P(o)	20?, 24, 28, 31		Blackman 1980 [Great Britain] (anholocyclic populations)
		20		Blackman 1980 [Iran]
		20, 24		Blackman and Eastop 2015 [?] (from grass roots)
<i>G. utricularia</i> (Passerini, 1856)	P(c), P(o)	16, 17, 18?		Blackman 1980 (as <i>G. enagrostidis</i> (Passerini, 1860)) [Great Britain] (anholocyclic populations)
		18		Blackman 1980 (as <i>G. enagrostidis</i> (Passerini, 1860)) [Italy]*
<i>Geoica ?wertheimae</i> Brown & Blackman, 1994	P(c)	18		Blackman and Eastop 2015 [?] (holocyclic populations on <i>Pistacia palaestina</i>)

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Geocia</i> sp.	?	18		Blackman 1980 [Israel]
<i>Gootiella tremulae</i> Tullgren, 1925	P(c), ?P(o)	16		Blackman and Eastop 2015 [?]
<i>Hemipodaphis persimilis</i> Akimoto, 1983	P(c)	36		Blackman 1986 [Japan]
<i>Kaltenbachiella elsboltriae</i> (Shinji, 1936)	P(c)	32		Blackman 1986 [Japan]
<i>K. japonica</i> (Matsumura, 1917)	P(c)	16/15	XX/X0	Blackman 1986 [Japan]
		18/?		Akimoto 1985 [Japan]
<i>K. pallida</i> (Haliday, 1838)	P(c)	28		Blackman 1980 [Great Britain]
<i>K. spinosa</i> Akimoto, 1985	P(c)	18		Akimoto 1985 [Japan]
<i>Melaphis rhois</i> (Fitch, 1866)	P(c), P(o)	26		Blackman and Eastop 1994 [?]
<i>Mordwilkoja vagabunda</i> (Walsh, 1863)	P(c), ?P(o)	20		Harper and MacDonald 1966, Robinson and Chen 1969a [Canada]
<i>Neoprociphilus aceris</i> (Monell, 1882)	P(c), P(o)	14		Robinson and Chen 1969a [Canada]
<i>Pachypappa marsupialis lambersi</i> Aoki, 1976	P(c)	10		Blackman 1986 [Japan]
<i>P. rosettei</i> (Maxson, 1934)	P(c)	10		Robinson and Chen 1969a (as <i>Asiphum</i>) [Canada]
<i>P. sacculi</i> (Gillette, 1914)	P(c)	10		MacDonald and Harper 1965 (as <i>Asiphum</i>), Harper and MacDonald 1966 (as <i>Asiphum</i>) [Canada]
<i>P. tremulae</i> (Linnaeus, 1761)	P(c)	10		Kuznetsova and Shaposhnikov 1973 (as <i>Asiphum</i>) [St. Petersburg, Russia]
<i>P. warshavensis</i> (Nasonov, 1894)	P(c)	10		Blackman and Eastop 1994 [?]
<i>Pachypappa</i> sp.	?	10		Blackman 1980 (as <i>Asiphum</i>) [Iran] (from <i>Populus euphratica</i>)
<i>Paracletus cimiciformis</i> von Heyden, 1837	P(c), P(o)	16		Blackman 1980 [Israel]
<i>Paracolopha morrisoni</i> (Baker, 1919)	P(c), ?P(o)	10/8	2(X ₁ X ₂)/ X ₁ X ₂ 0	S. Akimoto, personal communication in Blackman 1986 (as <i>Colopha moriokaensis</i> (Monzen, 1923) [Japan], Blackman and Eastop 1994 [?]
<i>Patchiella reaumuri</i> (Kaltenbach, 1843)	P(c), P(o)	12		Colling, 1955 (as <i>Pachypappella</i>) [Great Britain]
<i>Pemphigus borealis</i> Tullgren, 1909	P(c)	20		Blackman and Eastop 1994 [?]
<i>P. bursarius</i> (Linnaeus, 1758)	P(c), P(o)	20		Baehr 1908, 1909 (as <i>P. pyriformis</i>) [Germany]
<i>P. dorocola</i> Matsumura, 1917	P(c)	20		Blackman 1986 [Japan]
<i>P. fuscicornis</i> (Koch, 1857)	P(o), ?P(c)	20/19	XX/X0	Kuznetsova and Shaposhnikov 1973 [Kiev, Ukraine], Kuznetsova 1974 [?]
<i>P. immunis</i> Buckton, 1896	P(c)	10		Pal and Khuda-Bukhsh 1982 [Srinagar, Jammu and Kashmir, India]
		20		Blackman and Eastop 2015 [?]
<i>P. ?laurifolia</i> Dolgova, 1973	P(c)	20		Blackman 1986 [Japan]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>P. matsumunui</i> Monzen, 1929	P(c)	12		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Blackman 1986 [Japan], Blackman and Eastop 1994 [?] ("an unusual chromosome number for a <i>Pemphigus</i> , confirmed for Japanese samples from <i>Thalictrum</i> ").
<i>P. microsetosus</i> Aoki, 1975	P(c)	22		Blackman 1986 [Japan]
<i>P. mordwilkoii</i> Cholodkovsky, 1912	P(c)	20		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India], Blackman and Eastop 1994 [?]
<i>P. passeki</i> Börner, 1952	P(c)	22		Gut 1976 [Holland]
<i>P. populicarius</i> Fitch, 1859	P(c)	20		MacDonald and Harper 1965 [Canada]
<i>P. populinigrae</i> (Schrank, 1801)	P(c), ?P(o)	22		Gut 1976 (as <i>P. filaginis</i> (Boyer de Fonscolombe, 1841) [Holland]
<i>P. populitransversus</i> Riley, 1879	P(c), ?P(o)	20		Harper and MacDonald 1966 [Canada]
<i>P. spyrothecae</i> Passerini, 1856	P(c)	20		Baehr 1909 [Germany]
<i>P. tartareus</i> Hottes & Frison, 1931	P(c)	20		Robinson and Chen 1969a (as <i>P. junctisensoriatus</i> Maxson, 1934) [Canada]
<i>Pemphigus</i> sp.	?	20		Blackman 1980 [USA] (from roots of <i>Euphorbia supina</i>)
<i>Prociphilus micheliae</i> Hille Ris Lambers, 1933	?	14		Kar et al. 1990 [India]
<i>P. osmanthae</i> Essig & Kuwana, 1918	P(c)	18		Khuda-Bukhsh and Kar 1990 [Shillong, Meghalaya, India]
<i>P. (Meliarhizophagus) fraxinifolii</i> (Riley, 1879)	P(c)	20		Robinson and Chen 1969a [Canada]
		22		Blackman and Eastop 1994 [?]
<i>P. (Paraprocephilus) baicalensis</i> (Cholodkovsky, 1920)	P(o), ?P(c)	12		Blackman 1986 [Japan], Blackman and Eastop 1994 [?]
<i>P. (P.) tessellatus</i> (Fitch, 1851)	P(c)	6		Blackman and Eastop 1994 [?]
<i>P. (Stagona) konoii</i> Hori, 1938	P(c)	18		Blackman 1986 [Japan], Blackman and Eastop 1994 [?]
<i>P. (S.) pini</i> (Burmeister, 1835)	P(c)	16		Blackman 1980 [Great Britain]
<i>P. (S.) xylostei</i> (De Geer, 1773)	P(c)	10		Pal and Khuda-Bukhsh 1983 [Garhwal, Uttarakhand, India], Blackman and Eastop 2015 [Europe]
<i>Prociphilus</i> sp. 1	?	18		Khuda-Bukhsh and Kar 1990 [Shillong, Meghalaya, India]
<i>Prociphilus</i> sp. 2	?	10		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>Rectinasus buxtoni</i> Theobald, 1914	P(c), P(o)	26		Blackman 1980 [Iran]
<i>Schlechtendalia chinensis</i> (Bell, 1851)	P(c)	c. 36		Blackman and Eastop 1994 [?]
<i>Smynthuroides betae</i> Westwood, 1849	P(c), P(o)	8		Blackman 1980 [Great Britain; Iran]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Tetraneura radicolica</i> Strand, 1929	P(c), ?P(o)	14		Blackman and Eastop 1984 [?]
		14/13	XX/X0	S. Akimoto, personal communication in Blackman 1986 [Japan]
<i>T. ulmi</i> (Linnaeus, 1758)	P(c), P(o)	14/13	XX/X0	Schwartz 1932 [Munich, Germany]
		14, 16		Galli and Manicardi 1998 (gall generation) [Italy]
		16		Blackman and Eastop 1984 [?]
<i>T. yezoensis</i> Matsumura, 1917	P(c), P(o)	12/11	XX/X0	S. Akimoto, personal communication in Blackman 1986 [Japan]
		12		Blackman and Eastop 1994 [Japan]
		18		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>T. (Tetraneurella) fusiformis</i> Matsumura, 1917	P(c), P(o)	18/16	2(X ₁ X ₂)/ X ₁ X ₂ 0	S. Akimoto, personal communication in Blackman 1986 [Japan]
		18		Blackman and Eastop 2015 [?] (gall generation)
		17, 18, 19, 20		Blackman and Eastop 2015 [?] (permanently parthenogenetic populations)
<i>T. (T.) nigriabdominalis</i> (Sasaki, 1899)	P(c), P(o)	14		Kulkarni and Kacker 1981a (as <i>T. hirsuta</i> Baker) [Sukna, West Bengal, India], Kulkarni 1984 [Darjeeling, West Bengal, India]
		14, 15, 16		Gautam et al. 1993, Manicardi and Gautam 1994 (as <i>T. akinire</i>) [Modena, Italy]
		17		Blackman and Eastop 1984 [?] (one sample), Blackman and Eastop 1994 [?] (anholocyclic population)
		18		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Blackman 1986 [Japan]
		13-19 with modal number 18	XX/X0	Galli and Manicardi 1998 (gall generation) [Italy]
		19		Blackman and Eastop 1994 [?] (anholocyclic population)
<i>T. (T.)</i> sp. 1 prope <i>nigriabdominalis</i> (Sasaki, 1899)	?	24		Blackman 1986 [Japan]
<i>T. (T.)</i> sp. 2 prope <i>nigriabdominalis</i> (Sasaki, 1899)	?	22, 26		S. Akimoto, personal communication in Blackman 1986 [Japan]
<i>T. (T.) sorini</i> Hille Ris Lambers, 1970	P(c)	16/14	2(X ₁ X ₂)/ X ₁ X ₂ 0	S. Akimoto, personal communication in Blackman 1986 [Japan]
<i>Tetraneura</i> sp.	?	10		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Thecabius affinis</i> (Kaltenbach, 1843)	P(c), P(o)	28		MacDonald and Harper 1965 (as <i>Th. populiconduplifolius</i> (Cowen, 1895)), Harper and MacDonald 1966 (as <i>Th. populiconduplifolius</i>) [Canada]
		38		Blackman 1980 [Great Britain], Blackman 1986 (as <i>Thecabius orientalis</i> (Mordvilko, 1935)) [Japan], Blackman and Eastop 2006 [British Columbia, Canada]
<i>T. (Parathecabius) auriculae</i> (Murray, 1877)	?	16		Blackman and Eastop 2006 [?]
<i>T. (P) latisensorius</i> (Hori, 1938)	P(c)	18+1 (1 B-chromosome?)		Blackman 1986 (as <i>Thecabius</i>) [Japan]
<i>T. (P) bysimachiae</i> Börner, 1916	P(c), P(o)	18		Gut 1976 [Holland]
Fam. Mindaridae				
<i>Mindarus abietinus</i> Koch, 1857	P(c)	12		Robinson and Chen 1969a [Canada], Blackman and Eastop 1994 [Europe]
<i>M. obliquus</i> (Cholodkovsky, 1896)	P(c)	8		Blackman and Eastop 1994: [?] (sample from <i>P. glauca</i> in British Columbia, Canada (leg. C.K. Chan) had 2n=8 (R.L. Blackman; unpublished data), indicating that there may be more than one species on <i>Picea</i> in Canada)
		12		Robinson and Chen 1969a [Canada]
Fam. Lachnidae				
<i>Cinara atlantica</i> (Wilson, 1919)	P(c), P(o)	10		Blackman and Eastop 1994 [?]
<i>C. atrotibialis</i> David & Rajasingh, 1968	?P(o)	10		Khuda-Bukhsh and Kar 1990 [Shillong, Meghalaya, India]
		22		Das et al. 1985 [India]
<i>C. braggii</i> (Gillette, 1917)	P(c)	10		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>C. cedri</i> Mimeur, 1936	P(c)	10		Blackman and Eastop 1994 [?]
<i>C. cembrae</i> (Seitner, 1936)	P(c)	10		Rukavishnikov 1979 [Siberia, Russia]
<i>C. coloradensis</i> (Gillette, 1917)	?	10		Robinson and Chen 1969a [Canada]
<i>C. confinis</i> (Koch, 1856)	P(c), P(o)	12		Blackman and Eastop 1994 [?]
<i>C. costata</i> (Zetterstedt, 1828)	P(c)	10		Blackman and Eastop 1994 [?]
<i>C. cronartii</i> Tissot & Pepper, 1967	?P(o)	10		Blackman and Eastop 1994 [?]
<i>C. cuneomaculata</i> (del Guercio, 1909)	P(c)	10/9		Shinji 1931 (as <i>Dilachmus laricis</i> (Walker, 1848)) [Japan]
<i>C. formosana</i> (Takahashi, 1924)	P(c)	10		Blackman and Eastop 1994 [?]
<i>C. fornacula</i> Hottes, 1930	P(c)	10		Robinson and Chen 1969a [Canada]
<i>C. hyperophila</i> (Koch, 1855)	P(c)	10		Rukavishnikov 1974, 1979 [Novosibirsk, Russia]
<i>C. kochiana</i> (Börner, 1939)	P(c)	10		Rukavishnikov 1974, 1979 (as <i>C. boernerii</i> Hille Ris Lambers, 1956 - see Mamontova 1991) [Novosibirsk, Russia], Blackman 1980 [Great Britain]
<i>C. lachnistrotris</i> Hille Ris Lambers, 1966	?	8		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>C. laricola</i> (Matsumura, 1917)	P(c)	10		Shinji 1927 (as <i>Dilachmus laricolus</i>), 1931, 1941a (as <i>C. laricis</i>), Blackman 1986 [Japan]
<i>C. laricifex</i> (Fitch, 1858)	P(c)	10		Robinson and Chen 1969a [Canada]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>C. laricis</i> Hartig 1839	P(c)	10		Rukavishnikov 1979 [Siberia, Russia]
<i>C. maculipes</i> Hille Ris Lambers, 1966	P(c)	12		Das et al. 1985 [Jammu and Kashmir, India], Kurl and Chauhan 1986a, 1987a [Chail, Himachal Pradesh, India]
<i>C. maghrebica</i> Mimeur, 1934	?	10		Blackman and Eastop 1994 [?]
<i>C. matsumurana</i> Hille Ris Lambers, 1966	P(c)	10		Blackman 1986 [Japan]
<i>C. nuda</i> Mordvilko, 1895	P(c)	10		Rukavishnikov 1974, 1979 [Novosibirsk, Russia]
<i>C. palaestinensis</i> Hille Ris Lambers, 1948	?P(o)	10		Blackman and Eastop 1994 [?]
<i>C. pectinatae</i> (Nördlinger, 1880)	P(c)	6		Blackman and Eastop 1994 [Germany] (2 samples) (about record of Rukavishnikov 1979 see <i>Cinara confinis</i> (Koch, 1856))
<i>C. pergandei</i> (Wilson, 1919)	P(c)	14		Blackman 1990 [?], Blackman and Eastop 1994 [?]
<i>C. piceae</i> (Panzer, 1801)	P(c)	10		Rukavishnikov 1979 (as <i>C. piceae</i> (Panzer, 1801) and also as misidentification of <i>C. pectinatae</i> (Nördlinger, 1880)) – see Mamontova 1991 [Novosibirsk, Russia], Blackman and Eastop 1994 [Great Britain]
<i>C. piceicola</i> (Cholodkovsky, 1896)	P(c)	8		Blackman 1990 [?], Blackman and Eastop 1994 [?]
<i>C. pilicornis</i> (Hartig, 1841)	P(c)	10		Blackman 1990, Blackman and Eastop 1994 [Great Britain; New Zealand]
		14		Rukavishnikov 1974, 1979 [Novosibirsk, Russia]
<i>C. pilosa</i> (Zetterstedt, 1840)	?	8		Blackman 1990 [?], Blackman and Eastop 1994 [?] (Mamontova 2001 noted 2n=14 according to Rukavishnikov 1979, but the last paper does not consider <i>C. pilosa</i> in reality)
<i>C. pinea</i> (Mordvilko, 1895)	P(c)	10		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
		10, 11, 14		Blackman 1990 [Great Britain]
		14		Rukavishnikov 1974, 1979 [Novosibirsk, Russia]
<i>C. pini</i> (Linnaeus, 1758)	P(c)	10/9	XX/X0	Rukavishnikov 1974, 1979 (as <i>C. pini</i> (Linnaeus, 1758) and as <i>C. hyperophila</i> (Koch, 1855) – see Mamontova 1991) [Novosibirsk, Russia], Blackman 1986 [Europe]
<i>C. pinidensiflorae</i> (Essig & Kuwana, 1918)	P(c)	10		Blackman and Eastop 2015 [?]
		22/21	XX/X0	Shinji 1931 (as <i>Dilachnus</i>), Blackman 1986 [Japan] (based on n(♂) = 11 (Shinji 1931))
<i>C. piniformosana</i> (Takahashi, 1923)	P(c)	10		Blackman 1986 [Japan]
<i>C. pinimaritimae</i> (Dufour, 1833)	P(c), ?P(o)	16		Blackman 1990, Blackman and Eastop 1994 (as <i>C. maritimae</i>) [?]
<i>C. ponderosae</i> (Williams, 1911)	P(c), P(o)	10		Blackman 1980 [USA]
<i>C. pruinosa</i> (Hartig, 1841)	P(c), P(o)	10		Blackman and Eastop 1994 [?]
<i>C. schimitscheki</i> Börner, 1940	P(c)	10		Blackman and Eastop 1994 [?]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>C. similis</i> (van der Goot, 1917)	?	12		Kulkarni and Kacker 1981a (as <i>Lachmus</i>) [Dadhau, Himachal Pradesh, India]
<i>C. strobi</i> (Fitch, 1851)	P(c)	10		Blackman and Eastop 1994 [?]
<i>C. tenuipes</i> Chakrabarti & Ghosh, 1974	?	12		Pal and Khuda-Bukhs 1982 (as <i>C. abieticola tenuipes</i> Chakrabarti and Ghosh) [Srinagar, Jammu and Kashmir, India] (probably misidentification – aphids were collected from unusual host plant, <i>Juniperus communis</i>)
<i>C. (Cupressobium) cupressi</i> (Buckton, 1881)	P(c), P(o)	12		Blackman 1980 [Great Britain]
<i>C. (C.) fresai</i> Blanchard, 1939	P(o)	13		Blackman 1980 [Great Britain]
<i>C. (C.) juniperi</i> (de Geer, 1773)	P(c)	12		Blackman 1980 [Great Britain]
<i>C. (C.) louisianensis</i> Boudreaux, 1949	?	12		Blackman 1990 [?]
<i>C. (C.) tujafilina</i> (del Guercio, 1909)	P(c)	12		Blackman 1980 [USA; Iran], Das et al. 1985 [India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>Essigella californica</i> (Essig, 1909)	P(c), P(o)	8		Blackman 1980 [USA]
<i>Eulachmus agilis</i> (Kaltenbach, 1843)	P(c)	8		Rukavishnikov 1979 (as <i>Protolachmus</i>) [Novosibirsk, Russia], Blackman 1980 [Great Britain; Sweden]
<i>E. brevipilosus</i> Börner, 1940	?P(o)	30		Blackman 1980 [Great Britain]
<i>E. rileyi</i> (Williams, 1911)	P(c), ?P(o)	8		Blackman 1980 [USA; Iran]
<i>E. thunbergii</i> (Wilson, 1919)	P(c)	8		Khuda-Bukhs and Kar 1990 [Shillong, Meghalaya, India]
		14/13	XX/X0	Shinji 1927, 1931 (as <i>E. piniformosanus</i> Takahashi, 1931), Blackman 1986 [Japan] (based on $n(\text{♂}) = 7$ (Shinji 1927, 1931))
<i>E. tuberculostemmatum</i> (Theobald, 1915)	?	8		Blackman 1986 [Europe], Khuda-Bukhs and Kar 1990 (cited after Blackman and Eastop 2015)
<i>Lachmus acutihirsutus</i> Kumar et Burkhardt, 1970	?	16		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>L. longirostris</i> (Mordvilko, 1909)	P(c)	8		Blackman 1990 (as <i>L. iliciphilus</i>) [West Germany]
<i>L. roboris</i> (Linnaeus, 1758)	P(c)	7?	2(X ₁ X ₂)/ X ₁ X ₂ 0	Blackman 1990 [West Germany]
		8 (7+1B)		Blackman 1990 [Czechoslovakia; West Germany]
		9 (7+2B)		Blackman 1990 [Czechoslovakia; Denmark; Poland]
		10		Blackman 1990 [Portugal; Great Britain?]
		11 (10+1B)		Blackman 1990 [Sweden; Great Britain]
		12?		Blackman 1990 [Portugal]
		14		Blackman 1990 [Great Britain]
		15 (13+ 2B?), 16, 17?		Blackman 1990 [Portugal]
		7, 8, 10, 11, 13, 16 and 17		Blackman and Eastop 2015 [?] (some of these may apply to <i>L. iliciphilus</i> ; a sample from <i>Castanea</i> in Portugal had 2n=10 (Blackman 1990))

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>L. tropicalis</i> (van der Goot, 1916)	P(c), ?P(o)	10	XX/X0	Shinji 1927, 1941a (as <i>Pterochlorus</i>), Blackman 1986 [Japan] (based on n(♂) = 5 (Shinji 1927))
		16		Shinji 1931 (as <i>Pterochlorus</i>) [Japan], Blackman 1986 [Japan] (based on n(♂) = 8 (Shinji 1931))
		12, 13 or 16		Blackman 1986, 1990 [Japan; China]
		12, 14, 16, 18, 22, 28, 38		Muramoto 1987 [Japan] (Blackman and Eastop 2015: " Muramoto (1987) reported chromosome numbers from 2n=14-38, but his results are difficult to interpret and may include polyploid cells and/or preparations of more than one species.")
<i>Maculolachmus sijkpensis</i> Hille Ris Lambers, 1962	P(c)	10		Robinson and Chen 1969a [Canada]
<i>M. submacula</i> (Walker, 1848)	P(c)	10		Blackman 1980 [Great Britain], Blackman and Spence 1996 [Great Britain]
		10/9	XX/X0	Blackman 1990 [Great Britain]
<i>Protrama flavescens</i> (Koch, 1857)	P(o)	40-42, c. 42		Blackman 1980 [Great Britain]
		~ 42, 42		Blackman et al. 2000 [Great Britain]
<i>P. radicis</i> (Kaltenbach, 1843)	P(o)	c.60		Blackman 1980 [Great Britain]
		~ 50		Blackman et al. 2000 [Great Britain]
<i>P. ranunculi</i> (del Guercio, 1909)	?	c.36		Blackman 1980 [Great Britain]
<i>Pterochloroides persicae</i> (Cholodkovsky, 1899)	P(c), P(o)	20		Blackman and Eastop 1984 [?], Blackman 1990 [?]
<i>Schizolachmus pineti</i> (Fabricius, 1781)	P(c), ?P(o)	10		Blackman 1980 [Great Britain]
		18		Rukavishnikov 1974, 1979 [Novosibirsk, Russia] (Blackman and Eastop 2015 supposed that the material from Novosibirsk may be misidentification of <i>S. obscurus</i>)
<i>Stomaphis bratislavensis</i> Czylok & Blackman, 1991	P(c)	8		Blackman 1990 (as <i>Stomaphis quercus</i> (Linnaeus, 1758)) [Czechoslovakia], Czylok and Blackman 1991 [Slovakia]
<i>S. cupressi</i> (Pintera, 1965)	?	14		Blackman 1990 [?]
<i>S. japonica</i> Takahashi, 1960	P(c)	10/8	2(X ₁ X ₂)/ X ₁ X ₂ 0	Blackman 1986 [Japan], Blackman 1990 [?], Czylok and Blackman 1991 [Japan]
<i>S. quercus</i> (Linnaeus, 1758)	P(c)	10/8	2(X ₁ X ₂)/ X ₁ X ₂ 0	Blackman 1990 [Europe]
<i>S. yanonis</i> Takahashi, 1918	P(c)	15, 16?	2(X ₁ X ₂)/ X ₁ X ₂ 0	Blackman 1990 [?]
		20?	2(X ₁ X ₂)/ X ₁ X ₂ 0	Honda 1921 (as <i>S. yanonis</i>), Blackman 1986 [Japan] (based on n(♂) = 10 (Honda 1921))
<i>Trama rara</i> Mordvilko, 1908	?	12		Blackman et al. 2000 [Great Britain]
		12, 13, 14		Normark 1999 [Great Britain; Poland]
		13		Blackman 1980 [Great Britain]
<i>T. troglodytes</i> von Heyden, 1837	P(o), P(c)	13, 14, 16, 17, 18, 19, 20, 21, 23		Normark 1999 [Great Britain; France; Germany; Czech Republic; Poland]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
		14, 15, 16, 17, 18, 19, 20, 21, 22		Blackman 1980 [Great Britain]
		14, 15, 17, 18, 19, 20, 21, 22, 23		Blackman et al. 2000 [Great Britain]
		16		Blackman et al. 2000 [Poland]
		16 (colony without sexual morphs), 20 (colony with sexual morphs)		Blackman et al. 2001 [Great Britain]
		21		Blackman and Spence 1996 [Great Britain]
<i>T. (Neotrama) caudata</i> del Guercio, 1909	P(o)	9, 11		Blackman 1980 (as <i>Neotrama</i>) [Great Britain]
		9, 10, 11, 12		Blackman et al. 2000 [Great Britain]
		10, 12		Normark 1999 [Great Britain]
<i>T. (N.) maritima</i> (Eastop, 1953)	P(o)	10, 11, 12, 13, 14		Normark 1999, Blackman et al. 2000 [Great Britain]
<i>Tuberolachnus salignus</i> (Gmelin, 1790)	P(o)	8		Morgan 1909b (as <i>Lachnus dentatus</i> Le Baron, 1872) [USA], Shinji 1927, 1931, 1941a (as <i>Tuberolachnus viminalis</i> (Fonscolombe)) [?] (based on n(♂) = 4 (Shinji 1927, 1931, 1941a), but Blackman 1980 supposed that all these data are misidentifications of different species of <i>Pterocomma</i>).
		20		Blackman 1986 [Japan], Blackman 1990 [Great Britain; Iran; India; Japan], Blackman and Spence 1996 [Great Britain]
		18, 19, 20		Dhatwalia and Gautam 2009 [Himachal Pradesh, India]
		22		Raychaudhuri and Das 1987 [India]
Fam. Hormaphididae				
<i>Aleurodaphis asteris</i> Takahashi & Sorin, 1958	P(o)	32		Blackman 1986 [Japan]
<i>A. impatientis</i> Sorin & Miyazaki, 2004	P(o)	c.30		Blackman and Eastop 2006 [?]
<i>A. mikaniae</i> Takahashi, 1925	?	c.30		Blackman 1986 [Japan]
<i>Astegopteryx bambusae</i> (Buckton, 1893)	?	12		Kar et al. 1990 [India]
<i>A. formosana</i> (Takahashi, 1924)	?	12		Chen and Zhang 1985b (as <i>A. insularis</i>) (cited after Blackman and Eastop 2015)
<i>A. himalayensis</i> (M.R. Ghosh, Pal & D.N. Raychaudhuri, 1977)		12		Kar et al. 1990 (as <i>Pseudoastegopteryx</i>) [India]
<i>A. minuta</i> (van der Goot, 1917)	?	12		Kar et al. 1990 [India]
<i>Cenataphis brasiliensis</i> (Hempel, 1901)	P(c), P(o)	18		Blackman and Eastop 2006 [?]
<i>C. orchidearum</i> (Westwood, 1879)	P(o)	16		Blackman and Eastop 1984, 2006 [?] (samples from <i>Cymbidium</i> , <i>Dendrobium</i> and <i>Epidendrum</i>)
		18		Blackman and Eastop 1984, 2006 [?] (samples from <i>Angraecum</i> , <i>Sarcocbilus</i> and <i>Butia</i>)

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Ceratoglyphina bambusae</i> van der Goot, 1917	P(c)	12		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>C. bengalensis</i> L.K. Ghosh, 1972	?	12		Khuda-Bukhsh and Kar 1987 (as <i>C. bambusae bengalensis</i> Ghosh) [Kalimpong, West Bengal, India]
<i>Cenatovacuna indica</i> M.R. Ghosh, Pal & D.N. Raychaudhuri, 1977	?	12		Kar et al. 1990 [India]
<i>C. japonica</i> (Takahashi, 1924)	P(c), P(o)	12		Blackman 1986 [Japan]
<i>C. lanigera</i> Zehntner, 1897	P(o)	12		Blackman and Eastop 1984 [?], Blackman 1986 [Japan], Kar et al. 1990 [India]
<i>C. nekoashi</i> (Sasaki, 1910)	P(c)	12		Blackman 1986 [Japan]
<i>C. perglandulosa</i> R.C. Basu, A.K. Ghosh & D.N. Raychaudhuri, 1975	?	12		Khuda-Bukhsh and Kar 1987 [Kalimpong, West Bengal, India]
<i>C. silvestrii</i> (Takahashi, 1927)	?	8		Kurl 1980b [Meghalaya, India]
		12		Khuda-Bukhsh and Kar 1987 [Kalimpong, West Bengal, India]
<i>Euthoracaphis umbellulariae</i> (Essig, 1932)	?P(c), P(o)	14		Blackman 1980 [USA]
<i>Hamamelistes betulinus</i> (Horvath, 1896)	P(o), P(c)	12		Kuznetsova and Shaposhnikov 1973 (as <i>Tetraphis</i>) [St. Petersburg, Russia], Blackman 1986 [Japan], Blackman and Eastop 2015 [?] (for anholocyclic European population)
<i>H. spinosus</i> Shimer, 1867	P(c)	c. 50		Blackman 1980 [Canada], Blackman and Eastop 1994 [?]
<i>Hormaphis betulae</i> (Mordvilko, 1901)	P(o), P(c)	?18		Blackman 1986 [Japan]
<i>H. cornu</i> (Shimer, 1867)	P(c)	?18		Blackman and Eastop 1994 [?]
<i>H. hamamelidis</i> (Fitch, 1851)	P(c)	?18		Blackman and Eastop 1994 [?]
<i>Pseudoregma alexanderi</i> (Takahashi, 1924)	P(o), ?P(c)	12		Khuda-Bukhsh and Kar 1987 (as <i>Paraoregma</i>) [Kalimpong, West Bengal, India]
<i>P. bambucicola</i> (Takahashi, 1921)	P(c), P(o)	12		Blackman 1986 [Japan], Chen and Zhang 1985b (cited after Blackman and Eastop 2015), Khuda-Bukhsh and Kar 1987 [Kalimpong, West Bengal, India]
<i>P. panicola</i> (Takahashi, 1921)	P(o)	12		Blackman 1986 [Japan]
<i>Thoracaphis</i> sp.	?	12		Blackman 1980 [Japan]
Fam. Thelaxidae				
<i>Glyphina betulae</i> (Linnaeus, 1758)	P(c)	10/9	XX/X0	Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia]
		28/27, 56/55	XX/X0	Blackman 1989 [Poland; Great Britain; Lithuania]
<i>G. jacutensis</i> Mordvilko, 1931	P(c)	8		Blackman 1989 [Romania; Lithuania]
		10		Kuznetsova and Shaposhnikov 1973 (as <i>G. schrankiana</i> Börner, 1950) [St. Petersburg, Russia]
<i>G. pseudoschrankiana</i> Blackman, 1989	P(c)	10/9	XX/X0	Blackman 1989 [Great Britain, Sweden]
<i>Glyphina</i> sp. from <i>Betula</i>	?	55		Blackman 1980 [Great Britain]
<i>Kwisakia onigurumii</i> (Shinji, 1923)	?P(c)	18		Blackman and Eastop 1994 [?] (or specimens from <i>Pterocarya stenoptera</i> in China)

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Thelexes californica</i> (Davidson, 1919)	P(c)	12		Blackman and Eastop 1994 [?]
<i>T. dryophila</i> (Schränk, 1801)	P(c)	8		Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia], Kuznetsova 1974 [?]
<i>T. suberi</i> (del Guercio, 1911)	?	8		Blackman and Eastop 1984 [?]
<i>T. valtadorosi</i> Remaudière, (1982) 1983	?	8		Blackman and Eastop 1994 [?]
Fam. Aiceonidae				
<i>Aiceona retipennis</i> David, Narayanan & Rajasingh, (1970) 1971	?	18		Khuda-Bukhsh 1980 [Garhwal, Uttarakhand, India]
Fam. Anoeciidae				
<i>Anoecia corni</i> (Fabricius, 1775)	P(c), P(o)	6		Blackman and Eastop 2015 [?]
		6, 7, 8 (rearrangements, hybridization?)		Blackman 1980 [Great Britain; Iran]
		8		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>A. cornicola</i> (Walsh, 1863)	P(c), P(o)	10		Robinson and Chen 1969a (as <i>A. querci</i> Fitch, 1859) [Canada]
<i>A. furcata</i> (Theobald, 1915)	P(o), ?P(c)	12		Gautam et al. 1993 [Modena, Italy]
		12, 13		Blackman 1980 (as <i>A. furcata</i> (Theobald, 1915) and as <i>A. nemoralis</i> Börner, 1950) [Great Britain]
<i>A. graminis</i> Gillette & Palmer, 1924	P(c)	8		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>A. haupti</i> Börner, 1950	P(c)	8		Blackman and Eastop 2015 [?]
<i>A. major</i> Börner, 1950	P(c)	7		Blackman 1980 [Great Britain] (2n=7 in possible hybrids with <i>corni</i>), Blackman and Eastop 2006 [?]
		8		Blackman 1980 [Great Britain], Blackman and Eastop 2006 [?]
<i>A. vagans</i> (Koch, 1856)	P(c)	12		Blackman 1980 [Great Britain; Sweden]
<i>Anoecia</i> sp. prope <i>haupti</i> Börner, 1950	?	8		Kuznetsova and Shaposhnikov 1973 [Crimea, Ukraine], Kuznetsova 1974 [?]
Fam. Phloeomyzidae				
<i>Phloeomyzus passerinii</i> Signoret, 1875	P(c), P(o)	10		Gut 1976 [Holland]
Fam. Greenideidae				
<i>Anomalosiphum indigoferae</i> A.K. Ghosh, M.R. Ghosh & D.N. Raychaudhuri, 1971	?	18		Blackman 1980 [Sarawak, Malaysia]
<i>Cervaphis quercus</i> Takahashi, 1918	?	8		Kurl 1980b [Meghalaya, India], Blackman 1986 [Japan]
<i>C. rappardi indica</i> A.N. Basu, 1961	?	8		Kar et al. 1990 [India]
<i>Eutrichosiphon heterotrichum</i> (Raychaudhuri, 1956)	P(c), P(o)	20		Blackman 1986 (as <i>E. dubium</i>) [Japan] (see Blackman and Eastop 2015)
<i>E. makii</i> Raychaudhuri & Chatterjee, 1974	?	40		Khuda-Bukhsh and Kar 1990 [Shillong, Meghalaya, India]
<i>E. parvulum</i> Eastop & Hille Ris Lambers, 1976	?	26		Blackman and Eastop 2006 [?]
<i>Eutrichosiphum</i> sp.	?	20		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Greenidea ayyari</i> D.N. Raychaudhuri, M.K. Ghosh, Banerjee, A.K. Ghosh, 1973	?	18		Gautam and Kumar 2006 [Shimla, Himachal Pradesh, India]
<i>G. ficicola</i> Takahashi, 1921	P(o)	22		Blackman 1980 [Australia]
<i>G. longisetosa</i> Raychaudhuri, Ghosh, Banerjee & Ghosh, 1973	?	18		Khuda-Bukhsh and Kar 1990 [Shillong, Meghalaya, India]
<i>G. mangiferae</i> Takahashi, 1925	?	20		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>G. querciphaga</i> Raychaudhuri, Ghosh, Banerjee & Ghosh., 1973	?	18		Gautam and Kumar 2006 [Shimla, Himachal Pradesh, India]
<i>G. (Trichosiphum) anonae</i> (Pergande, 1906)	?P(o), ?P(c)	22		Khuda-Bukhsh and Kar 1990 [Shillong, Meghalaya, India]
<i>G. (T.) bucktonis</i> A.K. Ghosh, R.C. Basu & D.N. Raychaudhuri, 1970	?	8		Kar et al. 1990 (as <i>G. (T.) schoutedeni</i> Raychaudhuri, Ghosh, Banerjee and Ghosh) [India]
		14		Kapoor and Gautam 1994 [Shimla, Himachal Pradesh, India]
<i>G. (T.) haldari</i> Maity & Chakrabarti, 1980	?	20		Gautam and Kumar 2006 [Shimla, Himachal Pradesh, India]
<i>G. (T.) heeri</i> D.N. Raychaudhuri, M.R. Ghosh, M. Banerjee & A.K. Ghosh, 1973	?	7, 8, 9		Kurl 1986 (as <i>G. (T.) formosana heeri</i> D.N. Raychaudhuri, M.R. Ghosh, M. Banerjee & A.K. Ghosh, 1973) [Meghalaya, India]
<i>G. (T.) kuwanai</i> (Pergande, 1906)	?P(c)	20		Blackman 1980, 1986 [Japan], Gautam and Kumar 2006 [Shimla, Himachal Pradesh, India]
<i>G. (T.) nipponica</i> Suenaga, 1934	P(c)	18		Blackman 1986 [Japan]
<i>G. (Trichosiphum) psidii</i> van der Goot, 1917	P(o)	18		Kulkarni and Kacker 1979 (as <i>G. (T.) formosana formosana</i> (Maki) [Rautara, West Bengal, India], Kar et al. 1990 (as <i>G. (T.) formosana formosana</i> (Maki) [India], Khuda-Bukhsh and Kar 1990 (as <i>G. (T.) formosana formosana</i> (Maki, 1917)) [Shillong, Meghalaya, India], Dutta and Gautam 1993 (as <i>G. (T.) formosana</i> (Maki, 1917)) [Mandi, Himachal Pradesh, India], Samkaria et al. 2010 (as <i>G. formosana</i> (Maki)) [Palampur, Himachal Pradesh, India]
<i>Mollitrichosiphum nandii</i> A.N. Basu, 1964	P(c)	16		Blackman and Eastop 1994 [?]
<i>Schoutedenia ralumensis</i> Rübsaamen, 1905	P(c), P(o)	14 (male)		Blackman 1980 [Australia]
		14		Khuda-Bukhsh and Kar 1990 (as <i>S. lutea</i> (van der Goot, 1917)) [Kalyani, West Bengal, India]
		15 (sex unknown)		Blackman and Eastop 1994 [Papua New Guinea]
		16/14	2(X ₁ X ₂)/ X ₁ X ₂ 0	Hales 1989 (as <i>S. lutea</i> (van der Goot)) [Australia]
Fam. Drepanosiphidae				
<i>Allaphis californica</i> (Hille Ris Lambers, 1974)	?	10		Blackman and Eastop 2006 (as <i>Thripsaphis</i>) [?]
<i>A. foxtonensis</i> (Cottier, 1953)	?	10		Blackman and Eastop 2006 (as <i>Thripsaphis</i>) [?]
<i>A. verrucosa</i> (Gillette, 1917)	P(c)	10		Blackman and Eastop 2006 (as <i>Thripsaphis</i>) [?]
<i>Betacallis alnicolens</i> Matsumura, 1919	?	22		Blackman 1986 [Japan]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>B. odaiensis</i> Takahashi, 1961	?	22		Blackman 1986 [Japan] provided these data and supposed that " <i>Eucenaphis betulifoliae</i> " in Shinji 1927 (with n=11) is very possibly <i>B. odaiensis</i> .
<i>B. sikkimensis</i> R.C. Basu, M.R. Ghosh & D.N. Raychaudhuri, 1974	P(c)	20		Khuda-Bukhs and Pal 1983b [Gharwal, Uttarakhand, India]
<i>Betulaphis brevipilosa</i> Börner, 1940	P(c)	20		Blackman and Eastop 1994 [?]
<i>B. pelei</i> Hille Ris Lambers, 1952	?	20		Blackman and Eastop 1994 [?]
<i>B. quadrituberculata</i> (Kaltenbach, 1843)	P(c)	20		Blackman 1980 [Sweden]
<i>Boernerina variabilis</i> Richards, 1961	P(c)	16		Blackman and Eastop 1994 [Canada]
<i>Calaphis arctica</i> Hille Ris Lambers, 1952	P(c)	18		Blackman and Eastop 1994 [?]
<i>C. betulaecolens</i> (Fitch, 1851)	P(c)	20		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>C. betulella</i> Walsh, 1863	P(c)	18		Blackman 1980 [USA]
<i>C. betulicola</i> (Kaltenbach, 1843)	P(c)	18		Gut 1976 [Holland]
<i>C. coloradensis</i> Granovsky, 1939	P(c)	18		Blackman 1980 [USA]
<i>C. flava</i> Mordvilko, 1928	P(c)	18		Gut 1976 [Holland], Blackman 1980 (as <i>C. viridipallida</i> Palmer, 1952) [Canada]
<i>C. leonardi</i> Quednau, 1971	P(c)	20		Blackman and Eastop 1994 [?]
<i>C. magnoliae</i> (Essig & Kuwana, 1918)	?	8/7	XX/X0	Shinji 1927, 1931, 1941a (as <i>Chromaphis</i>), Blackman 1986 (as <i>Neocalaphis</i>) [Japan] (based on n(♂) = 4 (Shinji 1931))
		14/13		Shinji 1941a [Japan] (Blackman 1986 supposed that "Shinji must have had immature males of another species")
		20		Blackman 1986 [Japan]
<i>C. magnolicolens</i> (Takahashi, 1921)	?	20/19	XX/X0	Shinji 1927, 1931, 1941a [Japan], Blackman 1986 (as <i>Neocalaphis</i>) [Japan] (their own data and based on n(♂) = 10 (Shinji 1927, 1931))
		20		Blackman 1986 [Japan]
<i>C. viridipallida</i> Palmer, 1952	P(c)	18		Blackman 1980 [Canada]
<i>Calaphis</i> sp.	?	18/17	XX/X0	Shinji 1927, 1931, 1941a (as <i>C. betulaecolens</i> Fitch, 1851) [USA]
<i>Callipterinella calliptera</i> (Hartig, 1841)	P(c)	20		Blackman 1980 [USA], Blackman 1986 [Japan]
<i>C. tuberculata</i> (von Heyden, 1837)	P(c)	20		Blackman 1980 [Great Britain]
<i>Chromaphis juglandicola</i> (Kaltenbach, 1843)	P(c)	8		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>Chromocallis nirecola</i> (Shinji, 1933)	P(c)	18		Blackman and Eastop 1994 [?]
<i>Clethrobius comes</i> (Walker, 1848)	P(c)	11 (structural heterozygote)		Blackman 1986 [Japan], Blackman 1988 [Japan; Great Britain; Ireland; Finland]
<i>Ctenocallis israelica</i> Hille Ris Lambers, 1954	?	16		Blackman and Eastop 2006 [?]
<i>C. setosa</i> (Kaltenbach, 1846)	P(c)	18		Blackman and Eastop 2006 [?]
<i>Drepanaphis acerifoliae</i> (Thomas, 1878)	P(c)	38		Shinji 1923 (as <i>Drefavaphis</i>) [USA]
		38/37	XX/X0	Shinji 1931 [USA]
<i>D. simpsoni</i> Smith, 1959	?	30		Blackman and Eastop 1994 [?]
<i>D. utabensis</i> Smith & Knowlton, 1943	?	30		Blackman 1980 [USA]
<i>Drepanosiphum braggii</i> Gillette, 1907	P(c)	30		Blackman 1980 [USA]
<i>D. iranicum</i> Hille Ris Lambers, 1971	P(c)	30		Blackman and Eastop 1994 [?]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>D. platanoidis</i> (Schrank, 1801)	P(c)	30		Shinji 1923 (as <i>Drefavosiphum flatavoides</i>) [USA]
		30/29	XX/X0	Shinji 1927, 1931, 1941a [USA]
<i>Eucalipterus tiliae</i> (Linnaeus, 1758)	P(c)	10		Blackman 1980 [Great Britain]
		10 (female), 8 (male)	2(X ₁ X ₂)/X ₁ X ₂ 0	Blackman and Eastop 1994 [?]
		38-40?		Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia]
<i>Euceraphis betulae</i> (Koch, 1855)	P(c)	10/8	2(X ₁ X ₂)/X ₁ X ₂ 0	Blackman 1976, 1977 [Great Britain], Blackman 1980 [Europe; West of North America], Blackman and Spence 1996 [Great Britain], Blackman and De Boise 2002 [Great Britain; New Zealand; USA]
		9, 10/7, 8		Blackman 1988 [Europe]
<i>E. betulae</i> group 1 (from <i>Betula papyrifera</i>)	?	7 (♀), 6 (♂)		Blackman 1980 [Northwest Territories, Canada], Blackman 1988 (Fig. 4b) [Northwest Territories, Yukon, Canada]
<i>E. betulae</i> group 2	?	8		Sun and Robinson 1966 (as <i>E. deducta</i> Baker, 1917), Robinson and Chen 1969a [Canada] (see comments in Blackman 1980)
<i>E. betulae</i> group 3	?	8 (+2) 2B-chromosomes		Blackman 1986 [Japan] (as <i>E. betulae</i>)
<i>E. betulijaponicae</i> (Matsumura, 1919)	P(c)	8 no B-chromosomes		Blackman 1986 [Japan] (as <i>E. betulae</i>)
		8(+1) 1B-chromosome 2n (♂) = 6 (+1) n (♂) = 4 (+1)		Blackman 1986 [Japan] (as <i>E. betulae</i>)
		9/7		Blackman and De Boise 2002 [Japan]
<i>E. borealis</i> Blackman, 2002	P(c)	8/7	XX/X0	Blackman 1980 (as <i>E. betulae</i> group) [Northwest Territories, Manitoba, Canada], Blackman and De Boise 2002 [Canada] (one pair of X-chromosome)
<i>E. caerulescens</i> Pashtshenko, 1984	P(c)	22		Blackman 1986 (as <i>E. ontakensis</i> Sorin, 1970), Blackman and De Boise 2002 [Japan]
<i>E. gillettei</i> Davidson, 1915	P(c)	15, 16, 18		Blackman 1980 [Canada; USA]
		15, 16, 18, 19/13, 17		Blackman 1988, Blackman and De Boise 2002 [Canada; USA]
<i>E. lineata</i> Baker, 1917	P(c)	16		Blackman 1980 (also as <i>E. deducta</i> Baker, 1917) [USA]
		16/14		Blackman 1988, Blackman and De Boise 2002 [USA]
<i>E. mucida</i> (Fitch, 1856)	P(c)	20		Blackman 1980 [New York, Pennsylvania, USA]
		20, 21, 22/18, 19, 20		Blackman 1988, Blackman and De Boise 2002 [USA] (the differences are due to variation in the number of accessory ("B") chromosomes)
<i>E. ontakensis</i> Sorin, 1970	?	22		Blackman 1986, 1988 [Japan]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>E. papyrifericola</i> Blackman, 2002	P(c)	9/7		Blackman and De Boise 2002 [Canada]
		9-10/8		Blackman 1980 [USA; Canada] (as <i>E. betulae</i> group) (the 2n=8 male record was probably due to misinterpretation of B-chromosomes in somatic cells, as all later males examined had 2n=7 (i.e. 2 X-chromosomes) – Blackman, personal comm.)
<i>E. punctipennis</i> (Zetterstedt, 1828)	P(c)	7, 8, 9		Blackman 1980 [Great Britain]
		7, 8 (without or with B chromosomes)		Blackman 1976 [Great Britain]
		7, 8/5, 6		Blackman 1988 [Europe]
		8/6		Blackman and De Boise 2002 [?]
		8		Blackman 1977 [?], Sun and Robinson 1966 [Canada]
<i>E. quedenai</i> Blackman, 2002	P(c)	11/9		Blackman 1980 [Utah, USA] (as <i>E. betulae</i> group), Blackman and De Boise 2002 [western USA] (including 3 "B" chromosomes)
<i>Hoplocallis picta</i> (Ferrari, 1872)	P(c)	14		Blackman and Eastop 2015 [?]
<i>Israelaphis carmini carmini</i> Essig, 1953	P(c)	18		Blackman 1980 (as <i>I. tavaresi</i> Ilharco, 1961) [Portugal]
<i>I. c. alistana</i> Mier Durante, 1978	P(c)	18		Blackman 1980 (as <i>I. tavaresi alistana</i> Mier Durante, 1978) [Spain]
<i>I. lambersi</i> Ilharco, 1961	P(c)	16		Blackman and Eastop 2006 [?]
<i>Melanocallis caryaefoliae</i> (Davis, 1910)	P(c)	14		Blackman 1980 (as <i>M. fumipennellus</i> (Fitch)) [USA]
<i>Mesocallis sawashibae</i> (Matsumura, 1917)	P(c)	10		Blackman 1986 (as <i>Pterocallis</i>) [Japan]
<i>M. (Paratinocallis) corylicola</i> (Higuchi, 1972)	?	10		Blackman 1986 (as <i>Pterocallis</i>) [Japan]
<i>Monaphis antennata</i> (Kaltenbach, 1843)	P(c)	20		Blackman and Eastop 2015 [?]
<i>Monellia caryella</i> (Fitch, 1855)	P(c)	18		Blackman 1980 [USA]
<i>M. microsetosa</i> Richards, 1960	P(c)	18		Blackman 1980 [USA]
<i>Monelliopsis caryae</i> (Monell, 1879)	P(c)	18		Blackman 1980 [USA]
<i>M. nigropunctata</i> (Granovsky, 1931)	P(c)	10		Blackman 1980 [Canada; USA]
<i>Myzocallis boernerii</i> Stroyan, 1957	P(c)	14		Blackman and Eastop 1994 [?]
<i>M. carpini</i> (Koch, 1855)	P(c)	14		Blackman and Eastop 1994 [?]
<i>M. coryli</i> (Goetze, 1778)	P(c)	14		Gut 1976 [Holland]
<i>M. glandulosa</i> Hille Ris Lambers, 1948	P(c)	14		Blackman and Eastop 1994 [?]
<i>M. (Agrioaphis) castanicola</i> Baker, 1917	P(c)	12/11		Shinji 1941a (as <i>Agrioaphis castanae</i>) [Japan]
		14	XX/X0	Kuznetsova et al. 1988 [St. Petersburg, Russia]
		14/13	XX/X0	Shinji 1923, 1927, 1931 (as <i>M. castanae</i> (Fitch, 1857)) [USA]
<i>M. (A.) myricae</i> (Kaltenbach, 1843)	P(c)	14		Gut 1976 [Holland]
<i>M. (Neodryomyzus) polychaeta</i> (David, 1969)	P(c)	12		Khuda-Bukhsh and Pal 1983b [Gharwal, Uttarakhand, India]
<i>M. (Neomyzocallis) discolor</i> (Monell, 1879)	P(c)	14		Robinson and Chen 1969a [Canada]
<i>M. (N.) punctata</i> (Monell, 1879)	P(c), P(o)	14		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>M. (Pasekia) cocciferina</i> Quednau & Barbaglio, 1991	?P(o)	14		Blackman and Eastop 1994 [?]
<i>M. (P.) komareki</i> (Pašek, 1953)	P(c)	14		Blackman and Eastop 1994 [?]
<i>Neochromaphis coryli</i> Takahashi, 1961	P(c)	18		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>Neophyllaphis araucariae</i> Takahashi, 1937	P(o), ?P(c)	18		Hales and Lardner 1988 [Australia]
<i>N. brimblecombei</i> Carver, 1971	P(c)	26/25		Hales and Lardner 1988 [Australia]
<i>N. gingerensis</i> Carver, 1959	P(c)	14		Hales and Lardner 1988 [Australia]
<i>N. grobleri</i> Eastop, 1955	P(c)	18		Hales and Lardner 1988 [Africa], Blackman and Eastop 1994 [?]
<i>N. lanata</i> Hales & Lardner, 1988	P(c)	24/23		Hales and Lardner 1988 [Australia]
<i>N. podocarpi</i> Takahashi, 1920	P(c), ?P(o)	24		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
		26		Blackman 1986 [Japan]
<i>N. totarae</i> Cottier, 1953	P(c)	10		Hales and Lardner 1988 [New Zealand], Blackman and Eastop 1994 [?]
<i>Neuquenaphis bulbicauda</i> Hille Ris Lambers, 1968	?	14	XX/X0	Blackman et al. 2003 [Chile]
<i>N. edwardsi</i> (Laing, 1927)	P(c)	12	XX/X0	Blackman et al. 2003 [Chile]
<i>N. palliceps</i> Hille Ris Lambers, 1968	?P(c)	6	XX/X0	Blackman et al. 2003 [Chile]
<i>N. schlingeri</i> Hille Ris Lambers, 1968	P(c)	12	XX/X0	Blackman et al. 2003 [Chile]
<i>N. sensoriata</i> Hille Ris Lambers, 1968	P(c)	16	XX/X0	Blackman et al. 2003 [Chile]
<i>N. similis</i> Hille Ris Lambers, 1968	?P(c)	14	XX/X0	Blackman et al. 2003 [Chile]
<i>N. staryi</i> Quednau & Remaudière, 1994	?	14	XX/X0	Blackman et al. 2003 [Chile]
<i>N. valdiviana</i> Carrillo, 1980	?	6	XX/X0	Blackman et al. 2003 [Chile]
<i>N. (Spicaphis) chilensis</i> Essig, 1953	?	10	XX/X0	Blackman et al. 2003 [Chile]
<i>N. (S.) essigi</i> Hille Ris Lambers, 1968	?	12	XX/X0	Blackman et al. 2003 [Chile]
<i>Neuquenaphis</i> sp. 1	?	12	XX/X0	Blackman et al. 2003 [Chile]
<i>Neuquenaphis</i> sp. 2	?	16	XX/X0	Blackman et al. 2003 [Chile]
<i>Oestlundia flava</i> (Davidson, 1912)	P(c)	8		Blackman 1980 [USA; Canada]
<i>Panaphis juglandis</i> (Goetze, 1778)	P(c)	22		Blackman and Eastop 1994 [?]
<i>Phyllaphis fagi</i> (Linnaeus, 1761)	P(c)	16		Blackman 1986 [Great Britain]
<i>P. fagifoliae</i> Takahashi, 1919	P(c)	26/25	XX/X0	Shinji 1931 (as <i>P. fagi</i> (Linnaeus, 1767) see Blackman 1986), Blackman 1986 [Japan] (based on n(♂) = 13 (Shinji 1931))
<i>Protopterocallis gigantea</i> Bissell, 1978	P(c)	10		Blackman 1980 [USA]
<i>Pterocallis abni</i> (De Geer, 1773)	P(c)	20		Blackman 1980 [USA]
<i>P. montana</i> (Higuchi, 1972)	?	16		Blackman 1986 [Japan]
<i>P. (Recticallis) nigrostriata</i> (Shinji, 1941)	P(c)	c. 26		Blackman and Eastop 1994 [?]
<i>Salusaphis scirpus</i> Theobald, 1915	P(c)	10		Blackman and Eastop 2015 [?]
<i>Sarucallis kahawauokalani</i> (Kirkaldy, 1907)	P(c)	6		Kurl 1978 (as <i>Neotherioaphis chhenafuli</i> Behura and Dash) [Meerut, Uttar Pradesh, India], Blackman 1980 [USA]
		8		Dutta and Khuda-Bukhsh 1980 (as <i>Tinocallis</i>) [Kalyani, West Bengal, India]
<i>Simochaitophorus maoui</i> Takahashi, 1936	P(c)	10		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>Sensoriaphis nothofagi</i> Cottier, 1953	P(c)	10		Blackman 1980 [New Zealand]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Shivaphis celti</i> Das, 1918	P(c), P(o)	6/5	XX/X0	Shinji 1927, 1931, 1941a, Blackman 1986 [Japan] (based on n(♂) = 3 (Shinji 1931))
		10		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Blackman 1986 [Hong Kong]
<i>Sh. (Sinishivaphis) hangzhouensis</i> (G. Zhang & Zhong, 1982)	?	10		Blackman and Eastop 1994 [?]
<i>Stegophylla essigi</i> Hille Ris Lambers, 1966	P(c), P(o)	12		Blackman 1980 [USA]
<i>S. quercina</i> Quednau, 1966	P(c)	> 30		Blackman and Eastop 1994 (as <i>Stegophylla quercicola</i> (Monell, 1879) [?])
<i>Strenaphis elongata</i> (Baker, 1917)	P(c)	10		Blackman and Eastop 2015 [?]
<i>Subsalsusaphis aquatilis</i> (Ossiannilsson, 1959)	?	8		Blackman and Eastop 2006 [?]
<i>S. flava</i> (Hille Ris Lambers, 1939)	P(c)	8		Blackman 1980 [Sweden]
<i>S. lambersi kamijiensis</i> Sorin, 2005	P(c)	6		Blackman and Eastop 2015 [?] ("Blackman 1980, erroneously listed as <i>S. saracola</i> ")
<i>S. ornata</i> (Theobald, 1927)	?	8		Gut 1976 [Holland]
<i>S. picta</i> (Hille Ris Lambers, 1939)	P(c)	10		Blackman 1980 [Sweden]
<i>S. virginica</i> (Baker, 1917)	P(c)	6		Blackman 1986 (as <i>S. saracola</i> Higuchi, 1972) [Japan]
<i>Symydobius alniarius</i> (Matsumura, 1917)	P(c)	20		Blackman 1986 [Japan]
<i>S. intermedius</i> Gillette and Palmer, 1930	P(c)	16		Blackman 1980 [USA]
<i>S. oblongus</i> (von Heyden, 1837)	P(c)	14 (male), 15 (female)		Blackman 1988 [Great Britain; Sweden; Czechoslovakia]
		16		Gut 1976 [Holland]
<i>S. (Yezocallis) kabae</i> (Matsumura, 1917)	P(c)	?26/25	XX/X0	Shinji 1931, 1941a, Blackman 1986 [Japan] (based on n(♂) = 13 (Shinji 1931))
<i>Takecallis arundicolens</i> (Clarke, 1903)	?P(c), ?P(o)	18		Blackman and Eastop 1984 [?], Blackman 1986 [Japan]
<i>T. arundinariae</i> (Essig, 1917)	P(o), ?P(c)	18		Blackman 1980 [USA; Great Britain], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Blackman 1986 [Great Britain], Khuda-Bukhsh and Kar 1990 [Shillong, Meghalaya, India]
<i>T. taiwana</i> (Takahashi, 1926)	?P(c), ?P(o)	16		Blackman and Eastop 1984 [?]
<i>Tamalia coweni</i> (Cockerell, 1905)	P(c)	6/5	XX/X0	Morgan 1915 (as <i>Phyllaphis</i>), Ris 1942 [USA]
<i>Therioaphis natricis</i> Hille Ris Lambers & van den Bosch, 1964	?	16		Blackman and Eastop 2006 [?]
<i>T. ononidis</i> (Kaltenbach, 1846)	P(c)	16		Blackman and Eastop 2006 [?]
<i>T. tenera</i> (Aizenberg, 1956)	P(c)	6		Blackman and Eastop 2006 [?]
<i>T. (Pterocallidium) trifolii trifolii</i> (Monell, 1882)	P(c), P(o)	16		Blackman 1980 [USA]
<i>T. (P.) t. maculata</i> (Buckton, 1899)	P(c), P(o)	16		Blackman 1980 [USA; Australia]
<i>T. (Rhizoberlesia) riehmii</i> (Börner, 1949)	P(c)	16		Robinson and Chen 1969a [Canada]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Thripsaphis ballii pennsylvanica</i> Quednau, 2010	?	8		Blackman and Eastop 2006 [?]
<i>Tiliaphis coreana</i> Quednau, 1979	P(c)	38		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>T. shinae</i> (Shinji, 1924)	P(c)	14/13	XX/X0	Shinji 1927, 1931, 1941a (as <i>Therioaphis</i>), Blackman 1986 [Japan] (based on n(♂) = 7 (Shinji 1931))
<i>Tinocallis ulmifolii</i> (Monell, 1979)	P(c)	8		MacDonald and Harper 1965 (as <i>Myzocallis</i>), Robinson and Chen 1969a [Canada]
<i>T. ulmiparvifoliae</i> Matsumura, 1919	P(c)	16		Blackman and Eastop 1994 [?]
<i>T. zelkowae</i> (Takahashi, 1919)	P(c)	12		Blackman 1986 (as <i>T. nirecola</i> (Shinji 1924) [Japan], Blackman and Eastop 1994 [?]
<i>T. (Sappocallis) saltans</i> (Nevsky, 1929)	P(c)	16		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>T. (S.) takachihoensis</i> Higuchi, 1972	P(c)	16		Blackman and Eastop 2015 [?]
<i>T. (S.) ulmicola</i> (Matsumura, 1919)	?	16		Blackman 1986 (as <i>Sappocallis</i>) [Japan]
<i>Tinocalloides montanus</i> Basu, 1970 (1969)	P(c)	18		Kurl 1981 [Shillong, Meghayala, India]
<i>Tuberculatus (Acanthocallis) quercicola</i> (Matsumura, 1917)	?	14/13	XX/X0	Shinji 1927, 1931, 1941a, Blackman 1986 [Japan] (based on n(♂) = 7 (Shinji 1931))
		16		Blackman 1986 [Japan], Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>T. (Acanthotuberculatus) radisectuae</i> G. Zhang, W. Zhang & Zhong, 1990	?	14		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>T. (Nippocallis) kuricola</i> (Matsumura, 1917)	P(c)	14/13	XX/X0	Shinji 1927, 1931 (as <i>Callipterus</i>) [Japan], Blackman 1986 (as <i>Myzocallis</i>) [Japan] (own data and based on n(♂) = 7 (Shinji 1931))
<i>T. (Orientuberculoides) capitatus</i> (Essig et Kuwana, 1918)	P(c)	14		Blackman and Eastop 2015 [?]
<i>T. (O.) kashiwae</i> (Matsumura, 1917)	P(c)	14/13	XX/X0	Shinji 1927, 1931 [Japan], Blackman 1986 [Japan] (own data and based on n(♂) = 7 (Shinji 1927, 1931))
<i>T. (O.) paranaracola hemitrichus</i> Hille Ris Lambers, (1972) 1974	?	14		Blackman and Eastop 2015 [?]
<i>T. (O.) yokoyamai</i> (Takahashi, 1923)	P(c)	14		Blackman 1986 [Japan]
<i>T. (Tuberculoides) annulatus</i> (Hartig, 1841)	P(c)	14		Blackman 1980 (as <i>Tuberculoides</i>) [Great Britain; USA]
<i>T. (T.) moerickei</i> Hille Ris Lambers, (1972) 1974	?	14		Blackman and Eastop 2015 [?]
<i>Tuberculatus</i> sp.	?	14		Kar et al. 1990 [India]
<i>Yamatocallis takagii</i> (Takahashi, 1963)	?	c. 48		Blackman 1986 [Japan]
Fam. Chaitophoridae				
<i>Atheroides hirtellus</i> Haliday, 1839	P(c)	8		Blackman 1980 [Great Britain]
<i>A. serrulatus</i> Haliday, 1839	P(c)	8		Blackman 1980 [Sweden]
<i>Chaitophorus capreae</i> (Mosley, 1841)	P(c)	30		Blackman 1980 [Great Britain]
<i>Ch. dorocolus</i> Matsumura, 1919	P(c)	14		Shinji 1941a, Blackman 1986 [Japan] (based on n(♂) = 7 (Shinji 1941a)) (but see Blackman 1986 p. 77)
<i>Ch. euphaticus</i> Hodjat, 1981	P(c), ?P(o)	22		Blackman and Eastop 2015 [?]
<i>Ch. furcatus</i> Quednau ex Pintera, 1987	P(c)	16		Blackman and Eastop 1994 [?]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Ch. himalayensis</i> (Das, 1918)	?	18		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>Ch. indicus</i> A.K. Ghosh, M.R. Ghosh & D.N. Raychaudhuri, 1970	P(c)	18		Pal and Khuda-Bukhsh 1983 (as <i>Ch. manaliensis</i> Chakrabarti, 1975) [Garhwal, Uttarakhand, India], Dutta and Gautam 1993 (as <i>Ch. manaliensis</i> Chakrabarti, 1975) [Shimla, Himachal Pradesh, India]
<i>Ch. inouyei</i> Hille Ris Lambers, 1976	?	26		Blackman and Eastop 2015 [?]
<i>Ch. leucomelas</i> Koch, 1854	P(c)	4		Rubín de Celis and Ortiz 1993 [Lima, Peru]
		36		Blackman and Eastop 2015 [Israel]
		40		Blackman 1980 [Great Britain], Blackman and Eastop 2015 [Great Britain; South Africa]
<i>Ch. ?matsumurai</i> Hille Ris Lambers, 1960	?	14		Shinji 1927 (as <i>Ch. salicohus</i>), 1931 (as <i>Ch. saliniger</i>), Blackman 1986 [Japan] (based on $n(\♂) = 7$ (Shinji 1927, 1931, 1941))
<i>Ch. neglectus</i> Hottes & Frison, 1931	P(c)	12		Robinson and Chen 1969a [Canada]
<i>Ch. niger</i> Mordvilko, 1929	P(c)	30		Blackman and Eastop 2015 [?]
<i>Ch. nigrae</i> Oestlund, 1886	P(c)	24		Blackman and Eastop 1994 [?]
<i>Ch. nigrivus</i> Hille Ris Lambers, 1966	P(c)	18		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>Ch. populeti</i> (Panzer, 1801)	P(c)	10		Pal and Khuda-Bukhsh 1982 [Srinagar, Jammu and Kashmir, India]
		12		Blackman and Eastop 1994 [Iran; China]
		14		Shinji 1941a (as <i>Ch. populi</i>), Blackman 1986 [Japan] (based on $n(\♂) = 7$ (Shinji 1941a)) (but see Blackman 1986, p. 77)
<i>Ch. populialbae</i> (Boyer de Fonscolombe, 1841)	P(c)	28		Chen and Zhang 1985 (cited after Blackman and Eastop 2015)
		30		Blackman and Eastop 2015 [?]
<i>Ch. populicola</i> Thomas, 1878	P(c)	18, 28, 32		Blackman and Eastop 1994 [?]
<i>Ch. populifolii</i> (Essig, 1912)	P(c)	12		Robinson and Chen 1969a (also as <i>Ch. populifolii neglectus</i> Hottes and Frison, 1931) [Canada]
<i>Ch. saliapteris</i> Shinji, 1924	?	14/13	XX/X0	Shinji 1927, 1931, 1941a, Blackman 1986 [Japan] (based on $n(\♂) = 7$ (Shinji 1931)) (Blackman and Eastop 2015: "Shinji's record of $2n=14$ ($n=7$) should probably be applied to another species of <i>Chaitophorus</i> ")
		30		Blackman and Eastop 2015 [?]
<i>Ch. salicti</i> (Schränk, 1801)	P(c)	28		Blackman 1980 [Sweden]
<i>Ch. prope salijaponicus niger</i> (Mordvilko, 1929)	?	30		Kuznetsova and Shaposhnikov 1973 (<i>Ch. aff. niger</i> Mordv.) [Georgia; Turkmenistan]
<i>Ch. saliniger</i> Shinji 1924	P(c)	8		Shinji 1931, Blackman 1986 [Japan]
		14		Blackman 1986 [Japan] (based on $n(\♂) = 7$ (Shinji 1931))
<i>Ch. stevensis</i> Sanborn, 1904	?	14		Blackman and Eastop 2015 [?]
<i>Ch. tremulae</i> Koch, 1854	P(c)	18		Blackman and Eastop 1994 [?]
<i>Ch. truncatus</i> Hausmann, 1802	P(c)	30		Blackman and Eastop 2015 [?]
<i>Ch. viminalis</i> Monell, 1879	P(c)	9, 10, 11		Morgan 1909b [USA]
		18		Robinson and Chen 1969a [Canada]
<i>Chaitophorus</i> sp. 1 (from <i>Populus euphratica</i>)	?	22		Blackman 1980 [Iran]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Chaitophorus</i> sp. 2	?	26		Blackman 1986 [Japan]
<i>Periphyllus acericola</i> (Walker, 1848)	P(c)	18		Gut 1976 [Holland], Blackman and Eastop 1994 [?]
<i>P. aceris</i> (Linnaeus, 1761)	P(c)	16		Gut 1976 [Holland], Blackman and Eastop 1994 [?]
<i>P. californiensis</i> (Shinji, 1917)	P(c)	18		Blackman 1986 [Great Britain]
		20	XX/X0	Shinji 1927, 1931, 1941a (as <i>P. aceris</i>) [Japan] (but see Blackman 1986), Blackman 1986 [Japan] (based on n(♂) = 10 (Shinji 1931))
<i>P. conacinus</i> (Koch, 1854)	P(c)	18		Gut 1976 [Holland]
<i>P. hirticornis</i> (Walker, 1848)	P(c)	18		Gut 1976 [Holland], Blackman and Eastop 1994 [?]
<i>P. koelreuteriae</i> (Takahashi, 1919)	?	10		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
		18		Blackman and Eastop 1994 [?]
		20/19	XX/X0	Shinji 1931, Blackman 1986 [Japan] (based on n(♂) = 10 (Shinji 1931))
		22		Shinji 1927, 1941a [Japan]
<i>P. kuwanaii</i> (Takahashi, 1919)	?	18		Blackman and Eastop 1994 [?]
<i>P. lyropictus</i> (Kessler, 1886)	P(c)	18		Gut 1976 [Holland]
<i>P. negundinis</i> (Thomas, 1878)	P(c)	20		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>P. testudinaceus</i> (Ferni, 1852)	P(c)	18		Gut 1976 [Holland]
<i>Sipha flava</i> (Forbes, 1885)	P(c), P(o)	10		Mayo and Starks 1972 [USA]
<i>S. glyceriae</i> (Kaltenbach, 1843)	P(c)	10		Blackman and Eastop 2006 [?]
		12		Gut 1976 [Holland]
<i>S. (Rungisia) elegans</i> del Guercio, 1905	P(c)	6		Sun and Robinson 1966 (as <i>S. agropyrella</i> Hille Ris Lambers, 1939), Robinson and Chen 1969a (as <i>S. kurdjumovi</i> Mordvilko, 1921)[Canada]
<i>S. (R.) maydis</i> Passerini, 1860	P(c), ?P(o)	12		Blackman and Eastop 2015 [?]
Fam. Aphididae				
<i>Abstrusomyzus phloxae</i> (Sampson, 1939)	P(o)	18		Blackman and Eastop 2006 [?]
<i>Acaudinum centaureae</i> (Koch, 1854)	P(c)	10		Kuznetsova 1968 (as <i>A. dolichosiphon</i> Mordvilko, 1928) [St.Petersburg, Russia]
<i>Acyrtosiphon auriculae</i> Martin, 1981	P(c)	8		Martin 1981 [Great Britain]
<i>A. bidenticola</i> Smith, 1960	?	8		Blackman and Eastop 2006 [?]
<i>A. boreale</i> Hille Ris Lambers, 1952	P(c)	10		Blackman and Eastop 2015 [?]
<i>A. caraganae caraganae</i> (Cholodkovsky, 1907(1908))	P(c)	10		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>A. c. occidentale</i> Hille Ris Lambers, 1947	P(c)	10		Blackman and Eastop 2015 [?]
<i>A. ghanii</i> Eastop, 1971	P(c), P(o)	10		Kapoor and Gautam 1994 [Shimla, Himachal Pradesh, India] (Blackman and Eastop 2015: "...but aphid was possibly misidentified as host was <i>Medicago</i> "), Blackman and Eastop 2006 [?]
<i>A. gossypii</i> Mordvilko, 1914	P(c), P(o)	6		Blackman 1980 [Iran], Gautam and Dhatwalia 2003 [Shimla, Himachal Pradesh, India]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>A. ignotum</i> Mordvilko, 1914	?	14		Pal and Khuda-Bukhsh 1984 (as <i>Metopolophium</i>), Khuda-Bukhsh and Pal 1986b (as <i>Metopolophium</i>) [Jamunetri, Uttarakhand, India]
<i>A. sp. prope ignotum</i> Mordvilko, 1914	?	10		Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia]
<i>A. kondoi</i> Shinji, 1938	P(c)	10		Blackman 1980 [USA], Blackman 1986 [Japan]
<i>A. lactucae</i> (Passerini, 1860)	P(c)	16		Blackman and Eastop 1984 [?]
<i>A. loti</i> (Theobald, 1913)	P(c)	10		Blackman 1980 [Great Britain]
<i>A. macrosiphum</i> (Wilson, 1912)	P(c)	10		Blackman and Eastop 2015 [?]
<i>A. malvae malvae</i> (Mosley, 1841)	P(c)	10		Blackman 1980 (as <i>A. pelargonii</i> Kaltenbach, 1843) [Great Britain], Kurl and Chauhan 1987b [Barog, Himachal Pradesh, India]
		12		Kar et al. 1990 (as <i>Metopolophium (Metopolophium) malvae</i> (Mosley)) [India]
<i>A. m. poterii</i> Prior & Stroyan, 1964	P(c)	10		Blackman and Eastop 2015 [?]
<i>A. m. rogersii</i> (Theobald, 1913)	P(c)	10		Blackman and Eastop 2015 [?]
<i>A. pisivorum</i> G. Zhang, 1980	?	14		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>A. pisum</i> (Harris, 1776)	P(c)	8/7	XX/X0	Pagliai 1965, Manicardi, Bizzaro et al. 1991, Bizzaro et al. 2000 [Italia]
		8		Suomalainen 1933 (as <i>Macrosiphum pisi</i> (Kaltenbach, 1843)) [Finland], Colling 1955 [Great Britain], Sun and Robinson 1966, Harper and MacDonald 1968, Robinson and Chen 1969a [Canada], Kuznetsova and Shaposhnikov 1973 (as <i>Dactinotus basalis</i> Walker, 1948) [St. Petersburg, Russia], Kuznetsova 1974 (as <i>Dactinotus basalis</i> Walk.) [?], Blackman 1986 [Japan], Khuda-Bukhsh and Kar 1990 [Kalimpong, West Bengal, India], Kar et al. 1990 [India], Blackman and Spence 1996 [Great Britain], Gautam and Dhatwalia 2003 [Shimla, Himachal Pradesh, India]
<i>A. primulae</i> (Theobald, 1913)	?	16		Blackman and Eastop 2015 [?]
<i>A. rubi</i> Narzikulov, 1957	P(c)	10		Pal and Khuda-Bukhsh 1982, Khuda-Bukhsh and Pal 1986b (as <i>Metopolophium sonchifoliae</i> Raychaudhuri, Ghosh & Das, 1980) [Srinagar, Jammu and Kashmir, India]
		12		Kurl and Chauhan 1986c, Kurl and Chauhan 1987a (as <i>Metopolophium</i>) [Barog, Himachal Pradesh, India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>A. scariolae</i> Nevsky, 1929	?	18		Blackman and Eastop 1984 [?]
<i>Akkaia polygoni</i> Takahashi, 1919	P(c)	12		Shinji 1927, 1931 [Japan] (Blackman 1986 supposed that "Shinji's immature males were of another species of <i>Akkaia</i> ")
		24		Blackman 1986 [Japan]
<i>Akkaia</i> sp.	?	12/11	XX/X0	Shinji 1931 [Japan] (see comments of Blackman 1986)
<i>Aleurosiphon smilacifoliae</i> (Takahashi, 1921)	P(c)	8		Blackman 1986 [Japan]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Amphicercidus japonicus</i> (Hori, 1927)	P(c)	8		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>A. loniceriae</i> Maity & Chakrabarti, 1982	?	18		Khuda-Bukhsh and Pal 1983a [Garhwal, Uttarakhand, India] (Blackman and Eastop 2015: «this was probably an error»)
<i>A. tuberculatus</i> David, Narayanan & Rajasingh, 1970 (1971)	?	6		Chauhan and Kurl 1990 [Dachigam, Jammu and Kashmir, India]
		12		Pal and Khuda-Bukhsh 1984 [Jamunetri, Uttarakhand, India]
<i>Amphicercidus</i> sp.		8?		Khuda-Bukhsh 1980 [Garhwal, Uttarakhand, India] (Blackman and Eastop 2015: <i>Amphicercidus loniceriae</i> Maity and Chakrabarti)
<i>Amphorophora agathonica</i> Hottes, 1950	P(c)	14		Robinson and Chen 1969a [Canada]
<i>A. ampullata ampullata</i> Buckton, 1876	P(c)	12		Blackman 1980, 2010 [Great Britain], Blackman 1986 [Japan]
<i>A. a. bengalensis</i> Hille Ris Lambers & Basu, 1966	?	12		Kurl and Chauhan 1986a [Kandaghat, Himachal Pradesh, India], Kurl and Chauhan 1987a [Manali, Himachal Pradesh, India]
<i>A. a. laingi</i> (Mason, 1925)	?	12		Sun and Robinson 1966 (as <i>A. laingi</i> (Mason, 1925)), Robinson and Chen 1969a (as <i>A. laingi</i> (Mason, 1925)) [Canada]
<i>A. amurensis</i> (Mordvilko, 1919)	?	14		Blackman 1986 [Japan]
<i>A. forbesi</i> Richards, 1959	?	12		Blackman and Eastop 2015 [?]
<i>A. gei</i> (Börner, 1939)	P(c)	12		Blackman 1980 [Great Britain]
<i>A. idaei</i> (Börner, 1939)	P(c)	18		Blackman et al. 1977 [Europe], Blackman 1980 [Great Britain; Germany]
<i>A. pacifica</i> Hill, 1968	P(o)	18		Blackman 1980 [USA]
<i>A. parviflori</i> Hill, 1958	?	12		Blackman 1980 [USA; Canada]
<i>A. rossi</i> Hottes & Frison, 1931	P(c)	46		Blackman and Eastop 2006 [?]
<i>A. rubi</i> (Kaltenbach, 1843)	P(c), P(o)	20, 21		Blackman et al. 1977 [Europe], Blackman 1980 [Great Britain]
<i>A. rubitoxica</i> Knowlton, 1954	?	30		Blackman 1980 [USA; Canada]
<i>A. sensoriata</i> Mason, 1923	?	72		Blackman 1980 [USA; Canada]
<i>A. stachyophila</i> Hille Ris Lambers, 1966	?	12		Blackman 1980 [USA]
<i>A. stolonis</i> Robinson, 1974	P(c)	48		Blackman 1980 [Canada]
<i>A. tiguatensa</i> Hottes, 1933	?	40		Blackman and Eastop 2006 [?]
<i>A. tuberculata</i> Brown & Blackman, 1985	P(c)	4	XX/X0	Blackman 1985, Blackman and Hales 1986, Blackman and Spence 1996, Spence and Blackman 1998 [Great Britain]
<i>Amphorophora</i> sp.	?	10		Blackman and Eastop 2006, Blackman 2010 [populations on <i>Athyrium felix-femina</i> in Netherlands and Great Britain]
<i>Anuraphis catonii</i> Hille Ris Lambers, 1935	P(c)	22		Kuznetsova 1968 [Crimea, Ukraine]
		26		Kuznetsova 1975 [Crimea, Ukraine]
<i>A. farfae</i> (Koch, 1854)	P(c)	12		Kuznetsova 1968, 1975 [St. Petersburg, Russia], Kuznetsova 1974 [?]
<i>A. farfae diana</i> Shaposhnikov, 1974	P(o)	12		Kuznetsova and Shaposhnikov 1973 [Georgia], Kuznetsova 1974 [?]
<i>A. pyrillaseri</i> Shaposhnikov, 1950	P(c), P(o)	12		Kuznetsova 1968, 1975 [Crimea, Ukraine]
<i>A. subterranea</i> (Walker, 1852)	P(c), P(o)	22		Kuznetsova 1968 [St. Petersburg, Russia]
		26		Kuznetsova 1975 [St. Petersburg, Russia]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Anuromyzus cotoneasteris</i> (Shaposhnikov, 1959)	P(c)	12		Kuznetsova 1968 (as <i>Dysaphis</i> (<i>Anuromyzus</i>)) [Georgia]
<i>Aphidura pannonica</i> Szelegiewicz, 1967	?	12		Blackman 1980 [Greece]
<i>Aphis acaenovinae</i> Eastop, 1961	?	8		Blackman and Eastop 2006 [Australia] ("D.F. Hales, pers. comm.")
<i>A. achyranthi</i> Theobald, 1929	?	7		Kurl and Chauhan 1987a [Solon, Himachal Pradesh, India]
		8		Kurl and Chauhan 1986b [Solon, Himachal Pradesh, India]
<i>A. affinis</i> Del Guercio, 1911	P(c)	8		Pal and Khuda-Bukhsh 1982, Khuda-Bukhsh and Pal 1985 [Srinagar, Jammu and Kashmir, India], Gautam and Sharma 1990 [Himachal Pradesh, India], Dutta and Gautam 1993, Gautam and Dhatwalia 2003 [Shimla, Himachal Pradesh, India]
<i>A. amaranthi</i> Holman, 1974	?	8		Blackman 1980 [USA]
<i>A. armata</i> Hausmann, 1802	P(c)	8		Gut 1976 [Holland]
<i>A. asclepiadis</i> Fitch, 1851	?	8		Stevens 1906, 1909 [USA], Robinson and Chen 1969a [Canada], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>A. brunnea</i> Ferrari, 1872	?	8		Blackman and Eastop 2015 [?]
<i>A. carduella</i> Walsh, 1863	P(c)	8		Sun and Robinson 1966 (as <i>Aphis helianthi</i> Monell, 1879), Robinson and Chen 1969a (as <i>Aphis helianthi</i> Monell, 1879 and <i>A. kurosawella</i> Davis, 1919) [Canada]
<i>A. celastrii</i> Matsumura, 1917	P(c)	8		Blackman 1986 (as <i>A. citricola celastrii</i> Matsumura, 1917) [Japan]
<i>A. chloris</i> Koch, 1854	P(c)	8		Blackman and Eastop 2015 [?]
<i>A. clematidis</i> Koch, 1854	P(c)	8		Khuda-Bukhsh and Pal 1985 [Garhwal, Uttarakhand, India]
<i>A. clerodendri</i> Matsumura, 1917	?	8		Blackman and Eastop 2015 [?]
<i>A. near clerodendri</i> Matsumura, 1917	?	8		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>A. clinopodii</i> Passerini, 1861	P(c)	8		Blackman and Eastop 2015 [?]
		7,8		Kurl 1986 [Meghalaya, India]
<i>A. craccivora</i> Koch, 1854	P(o), P(c)	8		Kurl 1978 [Jodhpur, Rajasthan; Modinagar, Uttar Pradesh, India], Kulkarni and Kacker 1979 [Kolkata, West Bengal, India], Blackman 1980 [USA; Iran], Chen and Zhang 1985a, c (also as <i>A. robiniae</i> Machiati) [Beijing area, China] (Chen and Zhang 1985a cited after Blackman and Eastop 2015), Kurl and Chauhan 1986b [Himachal Pradesh, India], Kuznetsova and Sapunov 1985, 1987 [Russia], Kar and Khuda-Bukhsh 1989 [Kalimpong, West Bengal, India], Kar et al. 1990 [India], Sen and Khuda-Bukhsh 1992 [West Bengal, India], Dutta and Gautam 1993 [Kangra, Himachal Pradesh, India], Kapoor and Gautam 1994 [Himachal Pradesh, India], Bakhtadze et al. 2010 [Georgia]
		9		Blackman 1980 [Iran] (from <i>Lupinus</i>)

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>A. crepidis</i> (Börner, 1940)	P(c)	8		Blackman and Eastop 2015 [?]
<i>A. cytisorum cytisorum</i> Hartig, 1841	P(c)	8		Blackman 1980 [USA], Chen and Zhang 1985a (as <i>sophoricola</i> Zhang) [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>A. cytisorum sarothamni</i> Fransen, 1928	P(c)	8		Blackman and Eastop 2015 [?]
<i>A. epilobii</i> Kaltenbach, 1843	P(c)	8		Gut 1976 [Holland]
<i>A. eugeniae</i> van der Goot, 1917	?	8		Blackman 1980 [Philippines; Australia]
<i>A. fabae fabae</i> Scopoli, 1763	P(c)	8		Colling 1955 [Great Britain], Orlando 1965 [Italy], Kurl and Chauhan 1986b, 1987a [Kangra, Himachal Pradesh, India], Kuznetsova and Gandrabur 1991 [St. Petersburg, Russia], Dutta and Gautam 1993 [Solan, Himachal Pradesh, India], Kapoor and Gautam 1994 [Himachal Pradesh, India], Blackman and Spence 1996 [Great Britain], Rivi et al. 2009 [Italy] Jangra et al. 2014 [Jammu and Kashmir, India]
		8, 9		Panigrahy and Patnaik 1991 (as <i>A. citricola</i>) [Chatrapur, Odisha, India]
		8 (structural heterozygosity)		Blackman 1980 [anhelicic population in California, USA]
<i>A. f. evonymi</i> Fabricius, 1775	P(c)	8		Blackman and Eastop 2015 [?]
<i>A. f. mordvilko</i> Börner & Janich 1922	P(c)	8		Blackman 1980 [Great Britain], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015) Blackman and Eastop 2015 [?]
<i>A. farinosa</i> J.F. Gmelin, 1790	P(c)	6/5		Baehr 1908, 1912 (as <i>A. saliceti</i>) [Germany], Morgan 1909b (as <i>A. salicola</i> Gillette & Baker, 1895) [USA], Shinji 1941a (as <i>Aphis saliceti</i>) [Japan]
		6		Stevens 1906 [USA], Baehr 1909 (as <i>A. saliceti</i>) [Germany], Kuznetsova and Shaposhnikov 1973 [?], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
		6	XX/X0	Kuznetsova and Gandrabur 1991 [St. Petersburg, Russia]
<i>A. forbesi</i> Weed, 1889	P(c)	8		Blackman and Eastop 1984 [?]
<i>A. frangulae</i> Kaltenbach, 1845	P(c), ?P(o)	8		Blackman and Eastop 2015 [?]
<i>A. fukii</i> Shinji, 1922	?	8		Blackman 1986 [Japan]
<i>A. genistae</i> Scopoli, 1763	P(c)	8		Gut 1976 [Holland]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>A. gossypii</i> Glover, 1877	P(o), P(c)	8		Stevens 1906, 1909 [USA], Shinji 1927 [Japan], Robinson and Chen 1969a [Canada], Kurl 1978 [Jodhpur, Rajasthan; Modinagar and Meerut, Uttar Pradesh, India], Kulkarni and Kacker 1979 [Baruipur, West Bengal, India], Chattopadhyay and Raychaudhuri 1980 [Kolkata, West Bengal, India], Khuda-Bukhsh and Datta 1981a, b [India], Khuda-Bukhsh and Pal 1985 [Srinagar, Jammu and Kashmir, India], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Blackman 1986 [Japan] (their own data and based on $n(\♂) = 4$ (Shinji 1927)), Kurl and Chauhan 1986b [Himachal Pradesh, India], Khuda-Bukhsh and Kar 1989b, Kar et al. 1990 [India], Gautam and Sharma 1990 [Himachal Pradesh, India], Kar and Khuda-Bukhsh 1991a [Meghalaya, West Bengal, India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India], Gautam and Dhatwalia 2003 [Solan, Himachal Pradesh, India], Samkaria et al. 2010 [Palampur, Himachal Pradesh, India], Reeta Devi and Gautam 2012 [Kullu region, Himachal Pradesh, India]
		10		Shinji 1941a [Japan]
<i>A. healyi</i> Cottier, 1953	P(c)	8		Blackman and Eastop 2006 [New Zealand]
<i>A. hederæ hederæ</i> Kaltenbach, 1843	P(c)	8		Bakhtadze et al. 2010 [Georgia]
<i>A. hederæ pseudohederæ</i> Theobald, 1927	?	8		Blackman 1980 (as <i>A. hederæ</i> form <i>pseudohederæ</i> Theobald) [USA]
<i>A. borii</i> Takahashi, 1923	?	8		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>A. hyperici</i> Monell, 1879	P(c)	8		Blackman and Eastop 2015 [?]
<i>A. ichigo</i> Shinji, 1922	?	8		Blackman 1986 [Japan]
<i>A. idaei</i> van der Goot, 1912	P(c)	8		Blackman 1980 [Great Britain]
<i>A. ilicis</i> Kaltenbach, 1843	?P(c)	8		Blackman 1980 [Great Britain]
<i>A. kurosawai</i> Takahashi, 1921	?P(c)	8		Blackman 1986 [Japan], Kurl and Chauhan 1987a, 1987b [Solan, Himachal Pradesh, India]
<i>A. near kurosawai</i> Takahashi, 1921	?	8		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>A. lambersi</i> (Börner, 1940)	P(c)	8		Blackman and Eastop 2015 [?]
<i>A. longisetosa</i> Basu, 1969(1970)	?	6		Kurl and Chauhan 1987a, b (as <i>Aphis ruborum longisetosa</i> Basu) [Solan, Himachal Pradesh, India], Khuda-Bukhsh and Kar 1990 (as <i>Aphis ruborum longisetosa</i> Basu) [Shillong, Meghalaya, India]
		8		Khuda-Bukhsh 1982 (as <i>A. ruborum longisetosa</i>) [Mussoorie, Uttarakhand, India], Khuda-Bukhsh and Pal 1985 (as <i>A. ruborum longisetosa</i>) [Srinagar, Jammu and Kashmir, India]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>A. longituba</i> Hille Ris Lambers, 1966	?	8		Kar et al. 1990 (as <i>A. clematidis simlaensis</i> Kumar & Burkhardt) [India], Dutta and Gautam 1993 (as <i>A. clematidis simlaensis</i> Kumar & Burkhardt) [Shimla, Himachal Pradesh, India]
<i>A. loti</i> Kaltenbach, 1862	P(c)	8		Blackman 1980 [Great Britain]
<i>A. maculatae</i> Oestlund, 1887	P(c)	8		Robinson and Chen 1969a [Canada]
<i>A. nasturtii</i> Kaltenbach, 1843	P(c)	8		Dionne and Spicer 1957 (as <i>A. abbreviata</i> Patch), Sun and Robinson 1966, Robinson and Chen 1969a [Canada], Kurl and Chauhan 1986b [Himachal Pradesh, India], Kar and Khuda-Bukhsh 1989 [Kalimpong, West Bengal, India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India], Kapoor and Gautam 1994 [Himachal Pradesh, India], Gautam and Dharwalia 2003 [Shimla, Himachal Pradesh, India], Samkaria et al. 2010 [Yol, Himachal Pradesh, India]
<i>A. neogillettei</i> Palmer, 1938	P(c)	8		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>A. nerii</i> Boyer de Fonscolombe, 1841	P(o), P(c)	8		Kurl 1978 [Jodhpur, Rajasthan; Modinagar, Uttar Pradesh, India], Kulkarni and Kacker 1980 [India], Blackman 1980 [Great Britain], Chattopadhyay and Raychaudhuri 1980 [Kolkata, West Bengal, India], Khuda-Bukhsh and Datta 1981b [India], Khuda-Bukhsh and Pal 1985 [Garhwal, Uttarakhand, India], Kapoor and Gautam 1994 [Nahan, Himachal Pradesh, India]
<i>A. newtoni</i> Theobald, 1927	P(c)	8		Blackman 1980 [Great Britain]
<i>A. odinae</i> (van der Goot, 1917)	P(o), P(c)	8		Kurl 1980a (as <i>Toxoptera</i>) [Assam, Meghalaya, India], Pal and Khuda-Bukhsh 1980 (as <i>Toxoptera</i>) [Uttarakhand, India], Khuda-Bukhsh and Pal 1984a (as <i>Toxoptera</i>) [Triyuginarayan, Uttarakhand, India], Chen and Zhang 1985a (as <i>Toxoptera</i>) [Beijing area, China] (cited after Blackman and Eastop 2015), Blackman 1986 (as <i>Toxoptera</i>) [Japan], Kar and Khuda-Bukhsh 1989 (as <i>Toxoptera</i>) [Kalimpong, West Bengal, India], Kar et al. 1990 (as <i>Toxoptera</i>) [India]
		10		Shinji 1941a [Japan]
<i>A. oestlundii</i> Gillette, 1927	P(c)	8		Blackman and Eastop 2015 [?]
<i>A. paraverbasci</i> Chakrabarti (1976) 1977	?	8		Kurl and Chauhan 1986b, 1987a [Solan, Himachal Pradesh, India]
<i>A. parietariae</i> Theobald, 1922	P(c)	8		Blackman and Eastop 2015 [?]
<i>A. platylobii</i> Carver & White, 1970	?	8		Blackman and Eastop 2006 [New South Wales, Australia]
<i>A. polygonata</i> (Nevsky, 1929)	P(c)	8		Blackman and Eastop 2006 [?]
<i>A. pomi</i> De Geer, 1773	P(c)	8		Robinson and Chen 1969a [Canada], Kuznetsova and Shaposhnikov 1973 [Leningrad Prov., Russia], Gautam and Kumari 2003 [Shimla, Himachal Pradesh, India]
		7,8,9		Criniti et al. 2005 [Italy]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>A. punicae</i> Passerini, 1863	P(c), P(o)	8		Blackman and Eastop 1984 [?], Panigrahy and Patnaik 1987 [Chatrapur, Odisha, India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India], Gautam and Dhatwalia 2003 [Solon, Himachal Pradesh, India]
<i>A. rhamnifila</i> David, Narayanan & Rajasingh, 1971	?	8		Khuda-Bukhsh 1982 [Mussoorie, India]
<i>A. rubicola</i> Oestlund, 1887	P(c)	8		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>A. ruborum</i> (Börner & Schilder, 1931)	P(c)	8		Blackman 1980 [Great Britain], Bakhtadze et al. 2010 [Georgia]
<i>A. rumicis</i> Linnaeus, 1758	P(c)	8		Colling 1955 [Great Britain]
<i>A. salicariae</i> Koch, 1855	P(c)	8		Sun and Robinson 1966 (as <i>A. corniella</i> Hille Ris Lambers, 1935), Robinson and Chen 1969a (as <i>A. corniella</i> Hille Ris Lambers, 1935) [Canada]
<i>A. sambuci</i> Linnaeus, 1758	P(c), P(o)	8		Gut 1976 [Holland], Blackman 1986 [Europe; Japan], Manicardi et al. 1998 [Italy]
<i>A. sambuci</i> group	?	10		Shinji 1941a (as <i>A. sambuci</i> Linnaeus) [Japan]
		12		Shinji 1927, 1931 (as <i>A. sambuci</i> Linnaeus), Blackman 1986 [Japan] (based on n(♂) = 6 (Shinji 1927, 1931))
<i>A. sedi</i> Kaltenbach, 1843	P(c)	8		Blackman and Eastop 2015 [?]
<i>A. solanella</i> Theobald, 1914	P(c), ?P(o)	7		Blackman 1980 [Iran] (from <i>Solanum</i>)
		8		Blackman 1980 [Great Britain], Khuda-Bukhsh and Pal 1985 (as <i>A. fabae solanella</i> Theobald, 1914) [Garhwal, Uttarakhand, India], Kar and Khuda-Bukhsh 1989 (as <i>A. fabae solanella</i> Theobald) [Kalimpong, West Bengal, India], Gautam and Sharma 1990 [Himachal Pradesh, India], Kar et al. 1990 (as <i>A. fabae solanella</i> Theobald) [India], Dutta and Gautam 1993 (as <i>A. fabae solanella</i> Theobald, 1914) [Shimla, Himachal Pradesh, India], Gautam and Dhatwalia 2003 (as <i>A. fabae solanella</i>) [Shimla, Himachal Pradesh, India]
<i>A. spiraeicola</i> Patch, 1914	P(c), P(o)	8		Sun and Robinson 1966, Robinson and Chen 1969a [Canada], Kurl 1978 [Jodhpur, Rajasthan, India], Kulkarni and Kacker 1981a [Solon, Himachal Pradesh, India], Khuda-Bukhsh 1982 [Mussoorie, India], Khuda-Bukhsh and Pal 1985 [Garhwal, Uttarakhand, India], Kurl and Chauhan 1986a, b (as <i>A. citricola</i> van der Goot) [Himachal Pradesh, India], Panigrahy and Patnaik 1987 (as <i>A. citricola</i> van der Goot) [Chatrapur, Odisha, India], Kar and Khuda-Bukhsh 1989 (as <i>A. citricola</i> van der Goot) [Shillong, Meghalaya, India], Sen and Khuda-Bukhsh 1992 [West Bengal, India], Kapoor and Gautam 1994 [Nahan, Himachal Pradesh, India], Gautam and Dhatwalia 2003 (as <i>A. citricola</i> van der Goot) [Solon, Himachal Pradesh, India]
		10		Chen and Zhang 1985a (as <i>A. citricola</i> van der Goot) [Beijing area, China] (cited after Blackman and Eastop 2015)

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>A. spiraeophaga</i> F.P. Müller, 1961	P(c)	8		Blackman and Eastop 2015 [?]
<i>A. spiraeophila</i> Patch, 1914	P(c)	8		Robinson and Chen 1969a [Canada]
<i>A. subnitida</i> (Börner, 1940)	?	8		Blackman and Eastop 2015 [?]
<i>A. tanaxacicola</i> (Börner, 1940)	P(c)	8		Blackman 1980 [Great Britain]
<i>A. thaspis</i> Oestlund, 1887	?	8		Robinson and Chen 1969a [Canada]
<i>A. triglochinis</i> Theobald, 1926	P(c)	8		Blackman and Eastop 1984 [?], Turčinavičienė et al. 1997 [Lithuania]
<i>A. ulicis</i> Walker, 1870	P(c)	8		Blackman 1980 [Great Britain]
<i>A. umbrella</i> (Börner, 1950)	P(c), ?P(o)	6		Blackman and Eastop 2006 [Iran]
		7		Blackman 1980 [Iran]
		8		Blackman and Eastop 2006 [Israel; Cyprus; Italy; Great Britain]
<i>A. verbasci</i> Schrank, 1801	P(c)	8		Khuda-Bukhsh and Pal 1985 [Kalyaani, West Bengal, India]
<i>A. viburni</i> Scopoli, 1763	P(c)	8		Colling 1955 [Great Britain]
<i>A. violae</i> Shouteden, 1900	P(c)	8		Blackman and Eastop 2015 [?]
<i>A. (Bursaphis) epilobiaris</i> Theobald, 1927	P(c)	8		Blackman 1980 [Great Britain]
<i>A. (B.) fluviialis</i> Martin, 1982	?P(o)	9		Blackman and Eastop 2015 [?]
<i>A. (B.) grossulariae</i> Kaltenbach, 1843	P(c)	8		Turčinavičienė et al. 1997 [Lithuania]
<i>Aphis (B.) neomexicana</i> (Cockerell & Cockerell, 1901)	?	8		Robinson and Chen 1969a [Canada]
<i>A. (B.) oenotherae</i> Oestlund, 1887	?P(c), P(o)	8		Blackman and Eastop 2015 [?]
		10/9		Stevens 1905a, b, 1906, 1910 [USA]
<i>A. (B.) schneideri</i> (Börner, 1940)	P(c)	8		Turčinavičienė et al. 1997 [Lithuania]
<i>A. (B.) varians</i> Patch, 1914	P(c)	8		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>A. (Toxoptera) aurantii</i> Boyer de Fonscolombe	P(o), ?P(c)	8		Pagliai 1961 [Italy], Kurl 1980a [Assam, Meghalaya, India], Kar et al. 1990 [India], Kar and Khuda-Bukhsh 1991a [Jammu and Kashmir, Meghalaya, West Bengal, India], Gautam and Dhatwalia 2003 [Shimla, Himachal Pradesh, India], Samkaria et al. 2010 [Palampur, Himachal Pradesh, India]
		8, 9		Panigrahy and Patnaik 1991 [Chatrapur, Odisha, India]
<i>A. (T.) citricidus</i> (Kirkaldy)	P(o), P(c)	7, 8		Kurl 1980a [Assam, Meghalaya, India], Kurl 1986 [Meghalaya, India], Kar and Khuda-Bukhsh 1989 [Kalimpong, West Bengal, India], Kar et al. 1990 [India]
<i>A. (Toxopterina) vandergooti</i> (Börner, 1939)	P(c)	8		Kuznetsova and Shaposhnikov 1973 (as <i>Chomaphis</i>) [Leningrad Prov., Russia], Blackman 1980 (as <i>Toxopterina</i>) [Great Britain]
<i>Aphis</i> sp. 1	?	8		Robinson and Chen 1969a [Canada]
<i>Aphis</i> sp. 2	?	8		Robinson and Chen 1969a [Canada]
<i>Aphis</i> sp. 3	?	8		Kuznetsova and Shaposhnikov 1973 [Crimea, Ukraine]
<i>Aphis</i> sp. 4	?	10		Khuda-Bukhsh and Pal 1985 [Srinagar, Jammu and Kashmir, India]
<i>Aphis</i> sp. 5	?	8		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>Aphis</i> sp. 6	?	8		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>Aphis</i> sp. 7	?	10		Khuda-Bukhsh and Kar 1990 [Shillong, Meghalaya, India]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Aphis</i> sp. 8	?	8		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>Aphis</i> sp. 9	?	8		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>Aphis</i> sp. 10 (<i>A. gossypii</i> complex)	?	8		Dutta and Gautam 1993 [Solan, Himachal Pradesh, India], Kapoor and Gautam 1994 [Nahan, Himachal Pradesh, India]
<i>Aphthargelia symphoricarpi</i> (Thomas, 1878)	P(c)	14		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>Aspidaphis adjuvans</i> (Walker, 1848)	P(c)	12		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
		14,16		Blackman and Eastop 2006 [Cyprus; Israel; Iran]
<i>Aspidophorodon (Eoessigia) longicauda</i> (Richards, 1963)	?	20		Blackman and Eastop 2015 [?]
<i>Atarsos grindeliae</i> Gillette, 1911	P(c)	12		Robinson and Chen 1969a [Canada]
<i>Aulacophoroides hoffmanni</i> (Takahashi, 1937)	P(c)	14		Blackman and Eastop 2006 [?China]
<i>Aulacorthum cercidiphylli</i> (Matsmura, 1918)	?P(c)	12		Blackman 1986 [Japan]
<i>A. circicola</i> (Takahashi, 1923)	P(c)	10		Blackman 1986 [Japan]
<i>A. dorsatum</i> Richards, 1967	P(c)	12		Blackman and Eastop 2006 [?Western North America]
<i>A. flavum</i> F.P. Müller, 1958	P(c)	12		Blackman and Eastop 2006 [?]
<i>A. ibotum</i> (Essig & Kuwana, 1918)	?	14/13	XX/X0	Shinji 1927 (as <i>Macrosiphum ligustrumae</i>), 1931 (as <i>Macrosiphum</i>), Blackman 1986 [Japan] (based on n(♂) = 7 (Shinji 1927))
<i>A. linderae</i> (Shinji, 1922)	?P(c)	12		Shinji 1941b (as <i>Myzus</i>), Blackman 1986 [Japan] (based on n(♂) = 6 (Shinji 1941b))
<i>A. magnoliae</i> (Essig & Kuwana, 1918)	P(c)	12/11	XX/X0	Shinji 1931, 1941a (as <i>Amphorophora</i>) [Japan]
<i>A. munadachi</i> (Shinji, 1928)	?P(c)	10		Blackman and Eastop 2015 [?]
<i>A. myriopterani</i> (G. Zhang, 1980)	?	10		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>A. palustre</i> Hille Ris Lambers, 1947	?P(c), ?P(o)	34		Blackman and Eastop 2006 [?]
<i>A. phytolaccae</i> Miyazaki, 1968	?	10		Blackman and Eastop 2006 [Japan]
<i>A. sensoriatum</i> (David, Narayanan & Rajasingh, 1971)	?	18		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>A. smilacis</i> Takahashi, 1965	?	10		Blackman 1986 [Japan]
<i>A. solani</i> (Kaltenbach, 1843)	P(c), P(o)	9, 10, 11		Blackman 1980 [Great Britain; California, USA]
		10		Dionne and Spicer 1957 [Canada], Pagliai 1966 [Italy], Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia; Crimea, Ukraine], Kulkarni and Kacker 1980 [India], Pal and Khuda-Bukhsh 1980 [Triyugarayan, Uttarakhand, India], Blackman 1986 [Japan], Khuda-Bukhsh and Pal 1986b [Triyugarayan, Uttarakhand, India], Kapoor and Gautam 1994 [Nahan, Himachal Pradesh, India], Samkaria et al. 2010 [Shimla, Himachal Pradesh, India]
<i>A. speyeri</i> Börner, 1939	P(c)	10		Blackman 1980 [Iran]
<i>A. spinacaudatum</i> (Kumar & Burchardt, 1971)	?P(c)	12		Khuda-Bukhsh and Basu 1987 (as <i>A. magnoliae</i>) (cited after Blackman and Eastop 2015)

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Aulacorthum</i> sp. 1	?	12		Khuda-Bukhsh and Kar 1990 [Kalimpong, West Bengal, India]
<i>Aulacorthum</i> sp. 2	?	12		Samkaria et al. 2010 [Yol, Himachal Pradesh, India]
<i>Brachycaudus belicbrysi</i> (Kaltenbach, 1843)	P(c), P(o)	10, 11, 12, 13		Kurl 1986 [Meghalaya, India]
		12		Kuznetsova 1968 [Georgia], Kurl 1978 [Delhi, India], Pal and Khuda-Bukhsh 1980 [Gourikund, Uttarakhand, India], Kulkarni and Kacker 1981a [Dadhau, Himachal Pradesh, India], Raychaudhuri and Das 1987 [India], Gautam and Sharma 1990 [Himachal Pradesh, India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>B. spiraeae</i> Börner, 1932	P(c)	12		Gut 1976 [Holland]
<i>B. (Acaudus) klugkisti</i> (Börner, 1942)	P(c)	10		Blackman and Eastop 2015 [?]
<i>B. (A.) lychnidis</i> (Linnaeus, 1758)	P(c)	12		Kuznetsova 1968 [St.Petersburg, Russia]
<i>B. (A.) populi</i> (del Guercio, 1911)	P(c)	12		Blackman and Eastop 2015 [?]
<i>B. (Appelia) prunicola</i> Kaltenbach, 1843)	P(c)	12		Colling 1955 [Great Britain]
<i>B. (A.) prunifex</i> (Theobald, 1926)	P(c)	12		Blackman and Eastop 2015 [Great Britain]
<i>B. (A.) schwartzi</i> (Börner, 1931)	P(c)	12		Gut 1976 [Holland], Gautam and Dhatwalia 2003 [Shimla, Himachal Pradesh, India]
<i>B. (A.) tragopogonis tragopogonis</i> (Kaltenbach, 1843)	P(c) ?	12		Blackman and Eastop 1984 [?]
		11		Blackman and Eastop 1984 [Israel]
<i>B. (A.) t. setosus</i> (Kaltenbach, 1843)	?	12		Blackman and Eastop 2015 [Iran]
<i>B. (Mordvilkomemor) amygdalinus</i> (Schouteden, 1905)	P(c), P(o)	12		Kuznetsova 1968 [Georgia], Gautam and Kapoor 2002 [Una, Himachal Pradesh, India]
<i>B. (M.) rumexicolens</i> (Patch, 1917)	P(c)	12		Kurl and Chauhan 1987a [Barog, Himachal Pradesh, India]
<i>B. (M.) sedi</i> (Jacob, 1964)	P(c)	8		Blackman and Eastop 2006 [?]
<i>B. (Nevskyaphis) bicolor</i> (Nevsky, 1929)	P(o), ?P(c)	12		Blackman and Eastop 2015 [?]
<i>B. (N.) malvae</i> Shaposhnikov, 1964	?	12		Blackman and Eastop 2015 [?]
<i>B. (Prunaphis) cardui</i> (Linnaeus, 1758)	P(c)	10		Kuznetsova 1968 [Georgia], Blackman and Eastop 1984 [?]
<i>B. (P.) jacobi</i> Stroyan, 1957	P(c)	12		Gut 1976 [Holland]
<i>B. (Scrophulaphis) persicae</i> (Passerini, 1860)	P(o), ?P(c)	10		Blackman and Eastop 1984 [?]
<i>Brachycolus cerastii</i> (Kaltenbach, 1846)	P(c)	14/13	XX/X0	Gut 1976 [Holland]
<i>Brachycorinella asparagi</i> (Mordvilko, 1929)	P(c)	10		Blackman and Eastop 1984 [?]
<i>B. lonicerina</i> (Shaposhnikov, 1952)	P(c)	10		Blackman and Eastop 2006 [?]
<i>Brachyunguis calotropicus</i> Menon & Pawar, 1958	?	8		Kurl 1978, Kurl and Misra 1980, 1981 [Jodhpur, Rajasthan, India]
<i>B. harmalae</i> Das, 1918	P(c), ?P(o)	8		Blackman and Eastop 2015 [?]
<i>B. lycii</i> (Nevsky, 1928)	?	8		Blackman and Eastop 2006 [?]
<i>B. tamaricis</i> (Lichtenstein, 1885)	P(c)	8		Blackman and Eastop 2015 [?]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Brevicoryne brassicae</i> (Linnaeus, 1758)	P(c), P(o)	8, 9		Panigrahy and Patnaik 1991 [Chatrapur, Odisha, India]
		12, 14		Reeta Devi and Gautam 2012 [Kullu region, Himachal Pradesh, India]
		14		Kulkarni 1984 [Darjeeling, West Bengal, India]
		16/15	XX/X0	Cognetti 1961a, b, Cognetti and Cognetti-Varriale 1961, Pagliai 1962 [Italy]
		16		MacDonald and Harper 1965, Sun and Robinson 1966, Robinson and Chen 1969a [Canada], Kar et al. 1990 [India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India], Kapoor and Gautam 1994 [Himachal Pradesh, India], Gautam and Dhatwalia 2003 [Hamirpur, Himachal Pradesh, India], Giannini et al. 2003 [Italy]
<i>Capitophorus carduinus</i> (Walker, 1850)	P(c)	16		Blackman and Eastop 2015 [?]
<i>C. cirsiophagus</i> Takahashi, 1961	?P(c)	16		Blackman and Eastop 2006 (recorded as <i>C. elaeagni</i> in Blackman, 1986) [?]
<i>C. elaeagni</i> (Del Guercio, 1894)	P(c)	16		Robinson and Chen 1969a [Canada], Blackman 1986 [Japan]
<i>C. formosartemisiae</i> (Takahashi, 1921)	?	16		Blackman 1986 [Japan]
<i>C. hippophaes</i> (Walker, 1852)	P(c), ?P(o)	10		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>C. h. javanicus</i> Hille Ris Lambers, 1953	?	10		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Blackman 1986 [Japan], Kar et al. 1990 [India]
<i>C. horni</i> Börner, 1931	P(c)	16		Blackman 1980 [Great Britain]
<i>C. inulae</i> (Passerini, 1860)	?P(o)	16		Blackman and Eastop 2015 [?]
<i>C. mitegoni</i> Eastop, 1956	?P(c)	9		Kurl and Chauhan 1986a, 1987a [Manali, Himachal Pradesh, India]
<i>C. pakansus</i> Hottes & Frison, 1931	P(c)	16		Blackman and Eastop 1994 [?]
<i>Capitophorus</i> sp. [<i>?eniwanus</i> Miyazaki, 1971]	?	10		Blackman and Eastop 2006 [China, near Beijing]
<i>Casimira carbernae</i> (Eastop, 1961)	P(c)	8		Blackman and Eastop 2015 [?]
<i>Catamergus kickapoo</i> (Hottes & Frison, 1931)	P(c)	10		Robinson and Chen 1969a (as <i>Macrosiphum</i>) [Canada]
<i>Cavariella aegopodii</i> (Scopoli, 1763)	P(c), P(o)	10		Blackman 1980 [Great Britain; Iran], Dutta and Gautam 1993, Gautam and Dhatwalia 2003 [Shimla, Himachal Pradesh, India]
		8, 9, 10		Dhatwalia and Gautam 2009 [Himachal Pradesh, India]
<i>C. araliae</i> Takahashi, 1921	?P(c), P(o)	14		Blackman 1986 [Japan]
<i>C. archangelicae</i> (Scopoli, 1763)	P(c)	6		Blackman 1980 [Great Britain]
<i>C. borealis</i> Hille Ris Lambers, 1952	P(c)	6		Blackman and Eastop 1994 [?]
<i>C. cicutae</i> (Koch, 1854)	P(c)	10		Blackman 1980 [Iran]
<i>C. intermedia</i> Hille Ris Lambers, 1969	?P(c)	6		Blackman 1980 [Great Britain]
<i>C. japonica</i> (Essig & Kuwana, 1918)	P(c)	8		Blackman 1986 [Japan]
<i>C. konoii</i> Takahashi, 1939	P(c)	8		Blackman and Eastop 1984 [?], Blackman 1986 [Iceland]
<i>C. pastinacae</i> (Linnaeus, 1758)	P(c)	8		Gut 1976 [Holland]
<i>C. salicicola</i> (Matsumura, 1917)	P(c)	10		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>C. sericola</i> Shinji, 1927	?	8		Shinji 1927 [Japan]

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<i>C. theobaldi</i> (Gillette & Bragg, 1918)	P(c)	8, 10		Blackman 1980 [Great Britain]
<i>C. (Cavariella) aquatica</i> (Gillette & Bragg, 1916)	P(c)	8		Blackman and Eastop 2015 [?]
<i>C. (Cavariellinopicauda) oenanthi</i> (Shinji, 1922)	?	8/7	XX/X0	Shinji 1931, Blackman 1986 [Japan] (based on n(♂) = 4(Shinji 1931))
<i>Cavariella</i> sp. 1	?	10		Robinson and Chen 1969a [Canada]
<i>Cavariella</i> sp. 2	?	6		Kuznetsova 1978 [St.Petersburg, Russia]
<i>Cavariella</i> sp. 3	?	10		Khuda-Bukhsh 1980 [Gharwal, Uttarakhand, India]
<i>Cavariella</i> sp. 4	?	12		Kar et al. 1990 [India]
<i>Ceruraphis eriophori</i> (Walker, 1848)	P(c)	14		Kuznetsova and Gandrabur 1991 [St. Petersburg, Russia] (they also noted that 2n=8 in Kuznetsova 1968, 1974 was erroneous)
<i>Chaetomyzus</i> sp.	?	12		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>Chaetosiphon gracilicorne</i> David, Rajasingh & Narayanan, (1970) 1971	?P(c)	16		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>Ch. (Pentatrichopus) coreanum</i> (Paik, 1965)	P(c)	8		Blackman and Eastop 1984 [?], Blackman 1986 [Japan]
<i>Ch. (P) fragaefolii</i> (Cockerell, 1901)	P(o), ?P(c)	12, 13, 14, 15		Blackman et al. 1987 [Old World, North America]
		13, 14, 15		Blackman and Eastop 2015 [?]
		14		Blackman and Eastop 1984 [?]
<i>Ch. (P) jacobi</i> Hille Ris Lambers, 1953	P(o)	17		Blackman et al. 1987 [Western North America]
<i>Ch. (P) minor</i> Forbes, 1884	P(c)	12		Blackman et al. 1987 [Eastern North America]
<i>Ch. (P) tetraerhodum</i> (Walker, 1849)	P(c)	14		Blackman 1980 (as <i>Pentatrichopus</i>) [Great Britain]
		16		Blackman and Eastop 2006 [one sample from Australian Capital Territory, Australia]
<i>Ch. (P) thomasi</i> Hille Ris Lambers, 1953	P(c)	12		Blackman et al. 1987 [Western North America]
<i>Chomaphis mira</i> Mordvilko, 1928	?	8		Kuznetsova and Shaposhnikov 1973 [Voronezh, Russia]
<i>Coloradoa artemisiae</i> (Del Guercio, 1913)	P(c)	16		Robinson and Chen 1969a [Canada]
<i>C. bournieri</i> Remaudière & Leclant, 1969	P(o)	22		Blackman and Eastop 2006 [?]
<i>C. huculaki</i> Szelegiewicz, 1981	?	c.24		Blackman and Eastop 2006 [immature specimen from China]
<i>C. ponticae</i> (Börner, 1940)	?	16		Blackman and Eastop 2015 [?]
<i>C. rufomaculata</i> (Wilson, 1908)	P(o), ?P(c)	8		Panigrahy and Patnaik 1987 [Chatrapur, Odisha, India]
		8, 17		Panigrahy and Patnaik 1991 [Chatrapur, Odisha, India]
		18		Das et al. 1985 [India]
<i>C. santolinae</i> Hille Ris Lambers, 1948	?	20		Blackman and Eastop 2006 [?] (specimens from <i>Artemisia monosperma</i>)
<i>C. viridis</i> (Nevsky, 1929)	?	16		Blackman and Eastop 2006 [?]
<i>Corylobium avellanae</i> (Schrank, 1801)	P(c)	10		Blackman and Eastop 1984 [?]
<i>Cryptaphis bromi</i> Robinson, 1967	P(c)	16		Robinson and Chen 1969a [Canada]
<i>C. geranicola</i> (Shinji, 1935)	P(c)	14		Blackman 1986 [Japan]
<i>C. poae</i> (Hardy, 1850)	P(c)	16		Sun and Robinson 1966 [Canada]
		20		Blackman and Eastop 1984 [?]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Cryptomyzus alboapicalis</i> (Theobald, 1916)	P(c), P(o)	12		Blackman 1980 [Great Britain], Bašilova et al. 2008 [Lithuania]
<i>C. ballotae</i> Hille Ris Lambers, 1953	P(o), ?P(c)	12		Blackman 1980 [Great Britain]
<i>C. galeopsidis</i> (Kaltenbach, 1843)	P(c)	12		Blackman 1980 [Great Britain], Bašilova et al. 2008 [Lithuania]
<i>C. korschelti</i> Börner, 1938	P(c)	12		Bašilova et al. 2008 [Lithuania]
<i>C. leonuri</i> Bozhko, 1961	P(c)	12		Bašilova et al. 2008 [Lithuania]
<i>C. maudamanti</i> Guldemond, 1990	P(c)	12		Bašilova et al. 2008 [Lithuania]
<i>C. ribis</i> (Linnaeus, 1758)	P(c)	12		Sun and Robinson 1966, Robinson and Chen 1969a [Canada], Bašilova et al. 2008 [Lithuania]
<i>C. taoi</i> Hille Ris Lambers, 1963	P(c)	12		Blackman and Eastop 2015 [?]
<i>C. ulmeri</i> Börner, 1952	P(c)	12		Bašilova et al. 2008 [Lithuania]
<i>C. (Ampullosiphon) stachydis</i> (Heikenneimo, 1955)	P(c)	12		Blackman and Eastop 2006 [?]
<i>Cryptosiphum artemisiae</i> Buckton, 1879	P(c)	8		Blackman 1980 [Great Britain], Blackman 1986 [Japan]
<i>Delphiniobium canadense</i> (Robinson, 1968)	P(c)	20		Blackman and Eastop 2006 [?]
<i>D. yezoense</i> Miyazaki, 1971	P(c)	12		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>Diuraphis mexicana</i> (Baker, 1934)	P(o), ?P(c)	8		Blackman and Eastop 2006 [?]
<i>D. noxia</i> (Mordvilko ex Kurdjumov, 1913)	P(c), ?P(o)	10		Blackman 1980 [South Africa]
		10/9	XX/X0	Novotná et al. 2011 [Czech Republic]
<i>D. (Holcaphis) agrostidis</i> (Muddathir, 1965)	P(c)	12		Blackman 1980 (as Holcaphis) [Great Britain]
<i>D. (H.) frequens</i> (Walker, 1848)	P(c)	14		Gut 1976 [Holland]
<i>D. (H.) bolci</i> (Hardy, 1850)	P(c)	14		Gut 1976 [Holland]
<i>Dysaphis affinis</i> (Mordvilko, 1928)	P(c)	12		Kuznetsova 1968 [Georgia]
<i>D. angelicae</i> (Koch, 1854)	P(c)	12		Blackman and Eastop 2015 [?]
<i>D. anthrisci anthrisci</i> Börner, 1950	P(c)	12/11	XX/X0	Kuznetsova 1968 [St. Petersburg, Russia], Kuznetsova and Gandrabur 1991 [Ukraine]
		12		Gautam and Kapoor 2002 [Shimla, Himachal Pradesh, India]
<i>D. a. majkopica</i> Shaposhnikov, 1961	P(c)	12		Kuznetsova 1968 [North Caucasus, Russia], Kuznetsova 1974 [?]
<i>D. apiifolia</i> (Theobald, 1923)	P(o), P(c)	12		Blackman 1980 [Iran]
<i>D. chaerophyllina</i> Shaposhnikov, 1959	P(c)	12	XX/X0	Kuznetsova 1968 [North Caucasus, Russia], Kuznetsova 1974 [?]
<i>D. crataegi crataegi</i> (Kaltenbach, 1843)	P(c), P(o)	12		Blackman 1980 [Great Britain]
<i>D. crataegi heracleana</i> (Narzikulov, 1955)	?	12		Kuznetsova and Daniyarova 1980 [Kondara, Tajikistan]
<i>D. devector</i> (Walker, 1849)	P(c)	12/11	XX/X0	Kuznetsova and Gandrabur 1991 [St. Petersburg, Russia]
<i>D. foeniculus foeniculus</i> (Theobald, 1923)	P(o)	12		Blackman and Eastop 1984 [?], Gautam and Kapoor 2002 [Shimla, Himachal Pradesh, India]
<i>D. f. malidauci</i> Shaposhnikov, 1976	P(c)	12		Kuznetsova 1968 [Alma-Ata, Kazakhstan]
<i>D. hirsutissima</i> (Börner, 1940)	P(c)	12		Kuznetsova 1968 [St. Petersburg, Russia]
<i>D. narzikulovi</i> Shaposhnikov, 1956	P(c)	12		Kuznetsova and Daniyarova 1980 [Kondara, Tajikistan]

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<i>D. newskyi aizenbergi</i> (Shaposhnikov, 1949)	P(c)	12		Kuznetsova 1968 (as <i>D. aizenbergi</i> (Shaposhnikov, 1949)) [St.Petersburg, Russia]
<i>D. radicola</i> (Mordvilko, 1897)	P(c)	12		Kuznetsova 1968 [St.Petersburg, Russia], Blackman 1980 [Great Britain]
<i>D. rumecicola</i> (Hori, 1927)	P(c), P(o)	12		Kuznetsova and Daniyarova 1980 (as <i>D. emicis</i> Mim.) [Kondara, Tajikistan]
<i>D. tulipae</i> (Boyer de Fonscolombe, 1841)	P(o)	11, 12		Blackman 1980 [Great Britain]
<i>D. (Cotoneasteria) microsiphon</i> (Nevsky, 1929)	P(c)	12		Kuznetsova 1968 [Georgia]
<i>D. (Pomaphis) aucupariae</i> (Buckton, 1879)	P(c)	12		Blackman and Eastop 1994 [?], Blackman and Eastop 2006 [?]
<i>D. (P.) maritima</i> (Hille Ris Lambers, 1955)	P(c)	12		Blackman and Eastop 2006 [?]
<i>D. (P.) pavlovskyana</i> Narzikulov, 1957	P(c)	12		Khuda-Bukhsh and Pal 1983a [Garhwal, Uttarakhand, India] (apparently it is <i>Dysaphis indica</i> Chakrabarti & Medda, 1993)
<i>D. (P.) plantaginea</i> (Passerini, 1860)	P(c)	12		Kuznetsova 1968 (as <i>D. mali</i> (Ferrari, 1872) [Crimea, Ukraine], Blackman 1986 [Japan], Criniti et al. 2009 [Italy])
<i>D. (P.) pyri</i> (Boyer de Fonscolombe, 1841)	P(c)	12		Kuznetsova 1968 [Crimea, Ukraine]
<i>D. (P.) reamuri</i> Mordvilko, 1928	P(c)	12		Kuznetsova 1968 [Crimea, Ukraine]
<i>D. (P.) sorbi</i> (Kaltenbach, 1843)	P(c)	12		Kuznetsova 1968 [St.Petersburg, Russia]
<i>Dysaphis</i> sp.	?	12		Kuznetsova 1968 (as <i>D. crataegi</i> (Kaltenbach, 1843)) [Georgia]
<i>Elatobium abietinum</i> (Walker, 1849)	P(c), P(o)	18		Blackman 1980 [Great Britain]
<i>Elatobium</i> sp.	?	8		Khuda-Bukhsh and Kar 1990 [Shillong, Meghalaya, India]
<i>Ericaphis fimbriata</i> (Richards, 1959)	P(c)	14		Blackman and Eastop 2015 [?]
<i>E. gentneri</i> (Mason, 1947)	P(c)	18, 19, 20, 21, 23/17, 19		Blackman and Eastop 2015 [British Columbia, Canada]
<i>E. scammelli</i> (Mason, 1940)	P(c)	14		Blackman and Eastop 2015 [?]
<i>E. wakibae</i> (Hottes, 1934)	P(c)	12		Blackman and Eastop 2015 [?]
<i>Ericolophium holsti</i> (Takahashi, 1935)	?	22		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>E. itoe</i> (Takahashi, 1925)	?	18		Blackman and Eastop 2006 [?]
<i>Eucarazzia elegans</i> (Ferrari, 1872)	?	12		Gautam and Kapoor 2002 [Shimla, Himachal Pradesh, India]
<i>Eumyzus eastopi</i> Maity & Chakrabarti ex Maity, Bhattacharya & Chakrabarti, 1982	?	10		Khuda-Bukhsh and Pal 1986b [Triyuginarayan, Uttarakhand, India]
<i>E. gallicola</i> Takahashi, 1963	?	12		Blackman 1986 [Japan]
<i>E. impatiensae</i> (Shinji, 1924)	P(c)	10		Pal and Khuda-Bukhsh 1980, Khuda-Bukhsh and Pal 1986b [Triyuginarayan, Uttarakhand, India]
		12		Blackman 1986 [Japan]
<i>Gysoaphis oestlundii</i> Hottes, 1930	?	4		Sun and Robinson 1966 [Canada], Robinson and Chen 1969a [Canada]
<i>Hayburstia atriplicis</i> (Linnaeus, 1761)	P(c)	14		Sun and Robinson 1966 (as <i>Brachycolus</i>), Robinson and Chen 1969a [Canada], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Hyadaphis coriandri</i> (B. Das, 1918)	P(c), ?P(o)	12		Blackman and Eastop 2006 [?]
		13		Blackman 1980 [Iran]
		14		Kuznetsova and Shaposhnikov 1973 (as <i>Semiaphis tataricae</i> (Aizenberg, 1935) [St. Petersburg, Russia])
<i>H. foeniculi</i> (Passerini, 1860)	P(c)	12, 14		Blackman and Eastop 2006 [?] (one sample from <i>Foeniculum</i> had a mixture of 2n=12 and 2n=14 individuals)
		13		Blackman and Eastop 2006 [?] (one sample from <i>Lonicera</i> , a <i>foeniculi</i> × <i>passerini</i> hybrid?)
		14		Gut 1976 [Holland] (on <i>Conium maculatum</i>), Gautam and Kapoor 2002 [Una, Himachal Pradesh, India], Blackman and Eastop 2006 [?] (for samples of <i>H. foeniculi</i> from <i>Conium</i> and <i>Foeniculum</i>)
<i>H. passerinii</i> (del Guercio, 1911)	P(c), P(o)	12		Kuznetsova and Shaposhnikov 1973 [Crimea, Ukraine]
<i>H. tataricae</i> (Aizenberg, 1935)	P(c)	14		Blackman and Eastop 2015 [?]
<i>Hyadaphis</i> sp.	?	12		Blackman 1980 [Great Britain]
<i>Hyalomyzus raoi</i> Hille Ris Lambers, 1973	?	8		Khuda-Bukhsh and Kar 1990 [Shillong, Meghalaya, India]
<i>Hyalopteroides humilis</i> (Walker, 1852)	P(c)	16		Blackman 1980 [Great Britain]
<i>Hyalopterus amygdali</i> (Blanchard, 1840)	P(c)	10		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>H. pruni</i> (Geoffroy, 1762)	P(c)	10		Shibata 1941 [Japan], Robinson and Chen 1969a [Canada], Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia; Turkmenistan], Kuznetsova 1974 [?], Pal and Khuda-Bukhsh 1982, Khuda-Bukhsh and Pal 1984a [Srinagar, Jammu and Kashmir, India], Blackman 1986 [Japan]
<i>Hyperomyzus carduellinus</i> (Theobald, 1915)	P(o)	12		Kurl and Chauhan 1986a [Dharampur, Himachal Pradesh, India], Kurl and Chauhan 1987a [Naldehra, Himachal Pradesh, India], Gautam and Kapoor 2002 [Una, Himachal Pradesh, India]
<i>H. lactucae</i> (Linnaeus, 1758)	P(c), P(o)	12		Colling 1955 [Great Britain], Sun and Robinson 1966 (as <i>Nasonovia</i>), Robinson and Chen 1969a [Canada], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>H. lampsanae</i> (Börner, 1932)	P(c)	12		Blackman 1980 [Great Britain]
<i>H. (Hyperomyzella) rhinanthi</i> (Schouteden, 1903)	P(c)	12		Blackman and Eastop 2015 [?]
<i>H. (Neonasonovia) picridis</i> (Börner & Blunck, 1916)	P(c)	12		Blackman 1980 [Great Britain], Blackman and Eastop 1984 [?]
<i>H. (N.) ribiellus</i> (Davis, 1919)	P(c)	12		Sun and Robinson 1966 (as <i>Amphorophora</i>), Robinson and Chen 1969a (as <i>Kakimia ribiella</i> (Davis, 1919)) [Canada], Blackman and Eastop 1984 [?]
<i>Hysteroneura setariae</i> (Thomas, 1878)	P(c), P(o)	12		Robinson and Chen 1969a [Canada], Kurl 1986 [Meghalaya, India], Khuda-Bukhsh and Kar 1990 [Kalyani, West Bengal, India], Kapoor and Gautam 1994 [Shimla, Himachal Pradesh, India]
<i>Idiopterus nephrolepidis</i> Davis, 1909	P(o)	12		Blackman and Spence 1996 [Great Britain]
		13		Blackman 1980 [Great Britain]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Illinoia alni</i> (Mason, 1925)	P(c)	10		Blackman 1980 [Canada]
<i>I. azaleae</i> (Mason, 1925)	P(o), ?P(c)	10		Blackman and Eastop 1984 [?]
<i>I. lirioidendri</i> (Monell, 1879)	P(c)	10		Blackman 1980 [USA]
<i>I. morrisoni</i> (Swain, 1918)	P(o)	10		Blackman and Eastop 2015 [?]
<i>I. pepperi</i> (MacGillivray, 1958)	P(c)	22		Blackman and Eastop 2000 [?]
<i>I. richardsi</i> (MacGillivray, 1958)	?	10		Blackman 1980 [Canada]
<i>I. spiraeae</i> (MacGillivray, 1958)	?	10		Blackman and Eastop 2015 [?]
<i>I. subviride</i> (MacDougall, 1926)	?	10		Blackman and Eastop 2006 [?]
<i>I. wahnaga</i> (Hottes, 1952)	P(c)	10		Sun and Robinson 1966 (as <i>Masonaphis</i>), Robinson and Chen 1969a (as <i>Masonaphis</i>) [Canada]
<i>I. (Amphorinophora) crystleae</i> (Smith & Knowlton, 1939)	P(c)	16		Blackman and Eastop 2006 [?]
<i>I. (Masonaphis) lambersi</i> (MacGillivray, 1960)	P(c), P(o)	10		Gut 1976 (as <i>Masonaphis</i>) [Holland]
<i>I. (M.) menziesiae</i> (Robinson, 1969)	P(c)	10		Blackman and Eastop 2006 [?]
<i>I. (Oestlundia) davidsoni</i> Mason, 1925	?	12		Blackman 1980 [USA]
<i>I. (O.) maxima</i> (Mason, 1925)	P(c)	12		Blackman 1980 [Canada]
<i>I. (O.) rubicola</i> (Oestlund, 1886)	P(c)	12		Shinji 1931 (as <i>Amphorophora rubicola</i> (Oestlund) [?USA], Robinson and Chen 1969a (as <i>Masonaphis</i>) [Canada]
<i>Impatientinum asiaticum asiaticum</i> Nevsky, 1929	P(c)	16		Gut 1976 [Holland], Pal and Khuda-Bukhsh 1980 [Sonprayag, Uttarakhand, India], Khuda-Bukhsh and Pal 1986b [Gourikund, Uttarakhand, India], Kapoor and Gautam 1994 [Nahan, Himachal Pradesh, India]
<i>I. a. dalhousiensis</i> Verma, 1969	?P(c)	16		Kurl and Chauhan 1986a, 1987a [Meclodganj, Himachal Pradesh, India]
<i>I. balsamines</i> (Kaltenbach 1862)	P(c)	16		Blackman and Eastop 2015 [?]
<i>I. impatiens</i> (Shinji, 1922)	?P(c)	16		Blackman 1986 [Japan]
<i>Indoidiopterus geranii</i> (Chowdhuri, R.C. Basu, Chakrabarti, & D.N. Raychaudhuri, 1969)	P(c)	12		Pal and Khuda-Bukhsh 1980, Khuda-Bukhsh and Pal 1986b [Triyuginarayan, Uttarakhand, India]
<i>Indomasonaphis imulae</i> (A.K.Ghosh & Raychaudhuri, 1972)	P(c)	30		Kurl and Chauhan 1986c [Barog, Himachal Pradesh, India]
		32		Kurl and Chauhan 1987a [Barog, Himachal Pradesh, India]
<i>Indomegoura indica</i> (van der Goot, 1916)	P(c)	10		Blackman and Eastop 2006 [?]
		12		Shinji 1927 (as <i>Amphorophora indicum</i>), Blackman 1986 [Japan] (based on n(♂) = 6 (Shinji 1927))
<i>Liosomaphis atra</i> Hille Ris Lambers, 1966	?	17		Kurl and Chauhan 1987b [Barog, Himachal Pradesh, India], Kurl and Chauhan 1988 [India]
		18		Kurl and Chauhan 1987a [Barog, Himachal Pradesh, India]
<i>L. berberidis</i> (Kaltenbach, 1843)	P(c)	18		Blackman 1980 [Great Britain]
<i>L. himalayensis</i> A.N. Basu, 1964	?P(c)	18		Pal and Khuda-Bukhsh 1984 [Jamunetri, Uttarakhand, India], Dutra and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>Lipaphis erysimi</i> (Kaltenbach, 1843)	P(c)	10		Gut 1976 [Holland]
<i>L. fritzmuellerei</i> Börner, 1950	P(c)	10		Blackman and Eastop 2015 [?]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>L. pseudobrassicae</i> (Davis, 1914)	P(c), P(o)	6/5	XX/X0	Fox 1957 [Virginia, USA]
		8		Chen and Zhang 1985a, c (as <i>L. erysimi</i>) [Beijing area, China] (Chen and Zhang 1985a cited after Blackman and Eastop 2015), Kar and Khuda-Bukhsh 1991b (as <i>L. erysimi</i>) [Kalyani, West Bengal, India]
		8–9		Blackman and Eastop 2015 [?] (anholocyclic populations in most parts of the world have 2n=9)
		8, 10		Gautam and Kapoor 2002 (as <i>L. erysimi</i>) [Una, Himachal Pradesh, India]
		8, 9, 10		Feng and You 1988 (as <i>L. erysimi</i>) [Taiwan]
		9, 10		Kurl 1986 (as <i>L. erysimi</i>) [Meghalaya, India]
		10		Kurl and Misra 1981 (as <i>L. erysimi</i>) [Jodhpur, Rajasthan, India], Gautam and Sharma 1990 (as <i>L. erysimi</i>) [Himachal Pradesh, India], Gautam and Dhatwalia 2003 (as <i>L. erysimi</i>) [Shimla, Himachal Pradesh, India]
		4, 5, 6, 7, 8, 9, 10, 15, 18		Khuda-Bukhsh and Pal 1984b (as <i>L. erysimi</i>) [Kalyani, West Bengal, India]
<i>Longicaudus trirhodus</i> (Walker, 1849)	P(c)	12		Gut 1976 [Holland], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>Macchiatiella itadori</i> (Shinji, 1924)	P(c)	12/11	XX/X0	Shinji 1927, 1931, 1941a (as <i>Acaudus</i>), Blackman 1986 [Japan] (based on n(♂) = 6 (Shinji 1927, 1931))
<i>Macromyzus woodwardiae</i> (Takahashi, 1921)	P(o), ?P(c)	12		Blackman 1986 [Japan]
<i>Macrosiphoniella absinthii</i> (Linnaeus, 1758)	P(c)	12		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>M. artemisiae</i> (Boyer de Fonscolombe, 1841)	P(c)	12		Gut 1976 [Holland]
<i>M. dimidiata</i> Börner, 1942	P(c)	12		Blackman and Eastop 2006 [?]
<i>M. formosartemisiae</i> Takahashi, 1921	?P(c), P(o)	10		Pal and Khuda-Bukhsh 1980, Khuda-Bukhsh and Pal 1986b [Rambara, Uttarakhand, India]
<i>M. huaidensis</i> G. Zhang, 1980	?	12		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>M. kikungshana</i> Takahashi, 1937	P(c)	12		Pal and Khuda-Bukhsh 1980, Khuda-Bukhsh and Pal 1986b [Triyuginarayan, Uttarakhand, India]
<i>M. ludoviciana</i> (Oestlund, 1886)	P(c)	12		Robinson and Chen 1969a [Canada]
<i>M. millefolii</i> (De Geer, 1773)	P(c)	12		Gut 1976 [Holland]
<i>M. pseudoartemisiae</i> Shinji, 1933	?	10		Pal and Khuda-Bukhsh 1982, Khuda-Bukhsh and Pal 1986b [Srinagar, Jammu and Kashmir, India], Dutta and Gautam 1993 [Solan, Himachal Pradesh, India]
		12		Kar and Khuda-Bukhsh 1989 [Kalimpong, West Bengal, India] (Blackman and Eastop 2006: "perhaps this was misidentified yomogifoliae?")
<i>M. sanborni</i> (Gillette, 1908)	P(o)	10		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Chen and Zhang 1985b (cited after Blackman and Eastop 2015)

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>M. sanborni</i> (Gillette, 1908)	P(o)	12		Boschetti 1963 [Italia], Blackman and Eastop 2015 [many samples from Great Britain and India], Khuda-Bukhsh and Datta 1981b [India], Gautam and Sharma 1990 [Himachal Pradesh, India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India], Blackman and Eastop 2006 [one sample from China]
<i>M. sejuncta</i> (Walker, 1848)	P(c)	10		Blackman 1980 [Great Britain]
<i>M. subterranea</i> (Koch, 1855)	P(c)	12		Gut 1976 (as <i>Macrosiphoniella trimaculata</i> Hille Ris Lambers, 1938) [Holland]
<i>M. szalaymarzsoi</i> Szelegiewicz, 1978	?	12		Blackman and Eastop 2006 [?]
<i>M. tanacetaria</i> (Kaltenbach, 1843)	P(c)	12		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
		12/11	XX/X0	Kuznetsova and Gandrabur 1991 [St. Petersburg, Russia]
<i>M. tapuskae</i> (Hottes & Frison, 1931)	P(c)	12		Blackman and Eastop 2015 [?]
<i>M. yomogifoliae</i> (Shinji, 1922)	?	12		Kulkarni 1984 (as <i>Macrosiphum yamagopholiae</i> (Shinji)) [Darjeeling, West Bengal, India]
<i>M. (Asterobium) yomenae</i> (Shinji, 1922)	?	12		Shinji 1927 (as <i>Amphorophora</i>), Blackman 1986 [Japan] (based on n(♂) = 6 (Shinji 1927))
<i>M. (Chosoniella) myohyangsani</i> Szelegiewicz, 1980	?	12		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>M. (Ch.) spinipes</i> A.N. Basu, 1968	?	10		Kar et al. 1990 [India]
<i>M. (Phalangomyzus) antennata</i> Holman & Szelegiewicz, 1978	?	12		Blackman and Eastop 2006 [?]
<i>M. (P.) grandicauda</i> Takahashi & Moritsu, 1963	?	12		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>M. (P.) oblonga</i> (Mordvilko, 1901)	P(c)	12		Gut 1976 [Holland]
<i>M. (P.) persequens</i> (Walker, 1852)	P(c)	12		Gut 1976 [Holland]
<i>Macrosiphoniella</i> sp. 1	?	12		Samkaria et al. 2010 [Shimla, Himachal Pradesh, India]
<i>Macrosiphoniella</i> sp. 2	?	12		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>Macrosiphum albifrons</i> Essig, 1911	P(c)	10		Blackman 1980 [USA]
<i>M. californicum</i> (Clarke, 1903)	P(c)	10		Blackman 1980 [USA]
<i>M. centranthi</i> Theobald, 1915	P(c), ?P(o)	10		Blackman and Eastop 1984 [?]
<i>M. cholodkovskyi</i> (Mordvilko, 1909)	P(c)	10		Blackman and Eastop 2015 [?]
<i>M. claytoniae</i> Jensen, 2000	P(o)	16		Blackman and Eastop 2015 [?]
<i>M. clematifoliae</i> Shinji, 1924	P(c)	18		Blackman 1986 [Japan], Blackman and Eastop 2015 [?] (*the karyotype suggests that this species may be a <i>Sitobion</i> *)
<i>M. clydesmithi</i> Robinson, 1980	P(c)	16		Blackman and Eastop 2015 [?]
<i>M. cornifoliae</i> (Shinji, 1924)	?P(c)	14/13	XX/X0	Shinji 1927, 1931, 1941a, Blackman 1986 [Japan] (based on 2n male = 13 (Shinji 1931))
<i>M. corydalis</i> (Oestlund, 1886)	P(c)	10		Blackman and Eastop 2015 [?]
<i>M. creelii</i> Davis, 1914	?P(c)	10		Blackman and Eastop 2015 [?]
<i>M. daphnoidis</i> Börner, 1940	P(c)	10		Blackman and Eastop 2015 [?]
<i>M. dicentrae</i> Jensen & Chan, 2009	P(c)	16		Blackman and Eastop 2015 [?]
<i>M. equiseti</i> (Holman, 1961)	P(c)	16		Blackman and Eastop 2015 [?]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>M. euphorbiae</i> (Thomas, 1878)	P(c), P(o)	10/9	XX/X0	Lawson 1936 (as <i>M. solanifoliae</i> Ashmead, 1882) [USA], Dionne and Spicer 1957 (as <i>M. solanifoliae</i>) [Canada], Pagliai 1966 [Italy], Sun and Robinson 1966, Robinson and Chen 1969a [Canada], Gautam and Kapoor 2002 [Shimla, Himachal Pradesh, India], Monti et al. 2011 [Italy]
<i>M. euphorbiellum</i> Theobald, 1917	P(c)	10		Blackman 1980 (as <i>M. amygdaloides</i> Theobald, 1925) [Great Britain]
<i>M. funestum</i> (Macchiati, 1885)	P(c)	10		Blackman 1980 [Great Britain]
<i>M. gei</i> (Koch, 1855)	P(c)	10		Gut 1976 [Holland]
<i>M. geranii</i> (Oestlund, 1887)	?	10		Robinson and Chen 1969a [Canada]
<i>M. hamiltoni</i> Robinson, 1968	?	10		Robinson and Chen 1969a [Canada]
<i>M. hellebori</i> Theobald & Walton, 1923	P(c), P(o)	10		Gut 1976 [Holland]
<i>M. impatientis</i> Williams, 1911	P(c)	10		Blackman and Eastop 2015 [?]
<i>M. knautiae</i> Holman 1972	P(c)	12	XX/X0	Voronova et al. 2010 [Byelorussia]
<i>M. manitobense</i> Robinson, 1965	P(c)	10		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>M. mordvilkoii</i> Miyazaki, 1968	P(c)	10		Blackman 1986 [Japan]
<i>M. occidentalis</i> (Essig, 1942)	P(c)	16		Blackman and Eastop 2015 [?]
<i>M. opportunisticum</i> Jensen, 2012	P(c)	16		Blackman and Eastop 2015 [?]
<i>M. osmaroniae</i> Wilson, 1912	P(c)	16		Blackman and Eastop 2006 [?]
<i>M. pachysiphon</i> Hille Ris Lambers, 1966	?	18		Kurl 1980b [Meghalaya, India], Gautam and Kapoor 2002 [Shimla, Himachal Pradesh, India]
<i>M. pallidum</i> (Oestlund, 1887)	?	10		Robinson and Chen 1969a [Canada], Gautam & Dhatwalia 2003 [Shimla, Himachal Pradesh, India]
<i>M. parvifoliae</i> Richards, 1967	?	16		Blackman and Eastop 2015 [?]
<i>M. penfroense</i> Stroyan, 1979	?P(o)	10		Blackman and Eastop 2015 [?]
<i>M. ptericolens</i> Patch, 1919	P(c)	16		Blackman 1980 (as <i>Sitobion</i>) [Great Britain; USA]
<i>M. pteridis</i> Wilson, 1915	P(c)	16		Blackman and Eastop 2015 [?]
<i>M. pyrifoliae</i> MacDougall, 1926	?P(c)	10		Blackman and Eastop 2015 [?]
		11		Blackman and Eastop 2015 [?]
		12		Blackman and Eastop 1994 [?]
<i>M. rhamni</i> (Clarke, 1903)	P(c)	16		Blackman and Eastop 2015 [?]
<i>M. rosae</i> (Linnaeus, 1758)	P(c), P(o)	10		Stevens 1905b, 1906, 1909 (as <i>Aphis</i>) [USA], Hewitt 1906 (as <i>Aphis</i>) [Great Britain], Baehr 1909 (as <i>Aphis</i>) [Germany], Cognetti 1961a, b, Cognetti and Cognetti-Varriale 1961 Boschetti and Pagliai 1964, Pagliai 1966 [Italy], Khuda-Bukhsh 1980 [Garhwal, Uttarakhand, India], Raychaudhuri and Das 1987 [India], Kar and Khuda-Bukhsh 1989 [Kalimpong, West Bengal, India], Gautam and Dhatwalia 2003, Samkaria et al. 2010 [Shimla, Himachal Pradesh, India], Reeta Devi and Gautam 2012 [Kullu region, Himachal Pradesh, India]
				Kulkarni 1984 [Darjeeling, West Bengal, India]
				Stschelkanovzew 1904 (as <i>Aphis</i>) [Germany]
<i>M. stanleyi</i> Wilson, 1915	P(c)	16		Blackman and Eastop 1994 [?]
<i>M. stellariae</i> Theobald, 1913	?	10		Blackman 1980 [Great Britain]
<i>M. tenuicauda</i> Bartholomew, 1932	?	10		Blackman and Eastop 2006 [?]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>M. tinctum</i> (Walker, 1849)	P(o)	10		Blackman and Eastop 2015 [?]
<i>M. walkeri</i> Robinson, 1980	P(o), ?P(c)	16		Blackman and Eastop 2015 [?]
<i>M. willamettense</i> Jensen, 2000	P(c)	10		Blackman and Eastop 2006 [?]
<i>M. woodsiae</i> Robinson, 1980	?P(c)	16		Blackman and Eastop 2006 [?]
<i>M. (Neocorylobium) pseudocoryli</i> Patch, 1919	P(c)	10		Blackman and Eastop 1994 [?]
<i>M. (Unisitobion) perillae</i> (G. Zhang, 1988)	P(c)	18		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>Macrosiphum</i> sp.	?	10		Robinson and Chen 1969a [Canada]
<i>Matsumuraja capitophoroides</i> Hille Ris Lambers, 1966	?P(c)	14		Kurl and Chauhan 1986 [Manali, Himachal Pradesh, India]
<i>M. nuditerga</i> Hille Ris Lambers, 1965	?	14		Blackman and Eastop 2015 [?]
<i>M. rubea</i> Sorin, 1965	?	14		Blackman 1986 [Japan]
<i>M. rubi</i> (Matsumura, 1918)	P(c)	14		Blackman 1986 [Japan]
<i>M. rubifoliae</i> Takahashi, 1931	P(c), P(o)	14		Blackman 1986 [Japan]
<i>M. rubiphila</i> Takahashi, 1965	?	14		Blackman and Eastop 2015 [?]
<i>Matsumuraja</i> sp.	?	18		Blackman and Eastop 2006 [?]
<i>Megoura crassicauda</i> Mordvilko, 1919	?	10		Blackman and Eastop 2015 [?]
<i>M. dooarsis</i> (A.K. Ghosh & D.N. Raychaudhuri, 1969)	?	20		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>M. lespedezae</i> (Essig & Kuwana, 1918)	?	12/11	XX/X0	Shinji 1927, 1931 (as <i>Amphorophora</i>), Blackman 1986 [Japan] (based on 2n (♂) =6 (Shinji 1931))
		14		Kulkarni and Kacker 1980, 1981b [India], Blackman and Eastop 1984 [?], Shinji 1941a (as <i>Myzus lespedezae</i>), Blackman 1986 [Japan]
<i>M. viciae</i> Buckton, 1876	P(c)	10/9	XX/X0	Manicardi, Gautam et al. 1991 [Italy]
		10		Pagliai 1966, Orlando 1974, 1983 [Italy], Blackman 1986 [Japan]
<i>Melanaphis arundinariae</i> (Takahashi, 1937)	?	8		Khuda-Bukhsh and Pal 1984a [Triyugarayan, Uttarakhand, India], Kar and Khuda-Bukhsh 1989 [Shillong, Meghalaya, India]
<i>M. bambusae</i> (Fullaway, 1910)	P(c), P(o)	8		Blackman and Eastop 1984 [?], Blackman 1986 [Japan]
		10		Kuznetsova and Shaposhnikov 1973 [Sukhumi, Georgia], Kuznetsova 1974 [?]
		12		Kar et al. 1990 [India]
<i>M. donacis</i> (Passerini, 1861)	P(c)	8		Pal and Khuda-Bukhsh 1980, Khuda-Bukhsh and Pal 1984a [Ghangaria, Uttarakhand, India], Kuznetsova and Shaposhnikov 1973 (as <i>Longiunguis</i>) [Kara-Kala, Turkmenistan]
<i>M. japonica</i> (Takahashi, 1919)	P(c)	c.22		Blackman and Eastop 2006 [?]
<i>M. meghalayensis meghalayensis</i> D.N. Raychaudhuri & C. Banerjee, 1974	?	10		Pal and Khuda-Bukhsh 1980, Khuda-Bukhsh and Pal 1984a [Gobindoghat, Uttarakhand, India]
<i>M. pynaria</i> (Passerini, 1861)	P(c)	8		Kuznetsova and Shaposhnikov 1973 (as <i>Longiunguis</i>) [Crimea, Ukraine], Gautam and Dhatwalia 2003 [Shimla, Himachal Pradesh, India], Criniti et al. 2009 [Italy]
<i>M. sacchari</i> (Zehntner, 1897)	P(o), ?P(c)	8		Blackman 1980 [India], Blackman 1986 [Hong Kong]
		10		Khuda-Bukhsh and Kar 1990 [Kalyani, West Bengal, India]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>M. sorghi</i> (Theobald, 1904)	P(o), ?P(c)	8		Blackman and Eastop 2015 [?]
<i>Melanaphis</i> sp.	?	22		Blackman 1986 [Japan] (Blackman and Eastop 2015: "a record of 2n=22 for <i>M. sacchari</i> in Japan (Blackman 1986) is referable to another, undescribed species")
<i>Metopeurum fuscoviride</i> Stroyan, 1950	P(c)	8		Blackman 1980 [Great Britain]
<i>Metopolophium albidum</i> Hille Ris Lambers, 1947	P(c)	16		Blackman and Eastop 2006 [?]
<i>M. dirhodum</i> (Walker, 1849)	P(c), P(o)	16, 18		Rubín de Celis et al. 1997 [Brazil]
		18		Sun and Robinson 1966 (as <i>Acyrtosiphon</i>), Robinson and Chen 1969a [Canada], De Barro 1992 [Australia]
<i>M. fasciatum</i> Stroyan, 1982	P(o), ?P(c)	18		Blackman and Eastop 2015 [?]
<i>M. festucae festucae</i> (Theobald, 1917)	P(c), P(o)	16		Blackman and Eastop 1984 [?]
<i>M. f. cerealium</i> Stroyan, 1982	P(c), P(o)	16		Blackman and Eastop 1984 [?]
<i>M. friscum</i> Hille Ris Lambers, 1947	P(c)	16		Blackman 1980 [Great Britain]
<i>Metopolophium</i> sp.	?	16		Kar et al. 1990 [India] (possible <i>Acyrtosiphum</i>)
<i>Microlophium carnosum</i> (Buckton, 1876)	P(c)	16		Kuznetsova and Shaposhnikov 1973 (as <i>M. evansi</i> (Theobald, 1923) [Crimea, Ukraine]
		18		Robinson and Chen 1969a [Canada]
		20		Blackman 1980 [Great Britain], Blackman 2010 [?]
<i>M. rubiformosanum</i> (Takahashi, 1927)	?	12		Pal and Khuda-Bukhsh 1982, Khuda-Bukhsh and Pal 1986b (as <i>Acyrtosiphum</i>) [Srinagar, Jammu and Kashmir, India]
<i>Microlophium ?sibiricum tenuicauda</i> Hille Ris Lambers, 1949	?	18		Blackman 1980 [North America]
<i>Microlophium</i> sp.	?	16		Blackman and Eastop 2006 [?] ("This is possibly the species with 2n=16 from Crimea listed as <i>M. evansi</i> Theobald by Kuznetsova and Shaposhnikov (1973)), Blackman 2010 [Great Britain]
<i>Micromyzella filicis</i> (van der Goot, 1917)	?	36		Blackman and Eastop 2015 [New Zealand]
<i>Micromyzodium filicium</i> David, 1958	?	12		Kar et al. 1990 [India]
<i>M. spinulosum</i> Miyazaki, 1971	?	10		Blackman 1986 [Japan]
<i>Micromyzus nikkoensis</i> Miyazaki, 1968	?	12		Blackman 1986 [Japan]
<i>Microsiphum woronieckae</i> Judenko, 1931	P(c)	12		Blackman and Eastop 2006 [?]
<i>Muscaphis escherichi irae</i> (Shaposhnikov, 1963)	P(c)	12		Kuznetsova 1968 (as <i>Toxopteryella drepanosiphoides irae</i> Shaposhnikov, 1963) [St. Petersburg, Russia]
<i>Myzackaia verbasci</i> (Chowdhuri, R.C. Basu, Chakrabarti & D.N. Raychaudhuri, 1969)	?	12		Kurl and Chauhan 1986a, 1987a [Manali, Himachal Pradesh, India]
<i>Myzaphis bucktoni</i> (Jacob, 1946)	P(c)	13		Blackman and Eastop 2006 [one sample from Portugal]
<i>M. rosarum</i> (Kaltenbach, 1843)	P(c), P(o)	4		MacDonald and Harper 1965, Harper and MacDonald 1968 [Canada], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India], Reeta Devi and Gautam 2012 [Kullu region, Himachal Pradesh, India]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Myzus cerasi</i> (Fabricius, 1775)	P(c)	10		Sun and Robinson 1966, Robinson and Chen 1969a [Canada], Kurl and Chauhan 1986d, 1987a [Solani, Himachal Pradesh, India], Bizzaro et al. 1999 [Italy], Blackman and Eastop 2015 [European and North American populations]
<i>M. c. umefoliae</i> (Shinji, 1924)	P(c)	12		Khuda-Bukhsh and Pal 1986a (as <i>Myzus cerasi</i> , but Blackman and Eastop 2015 - "possibly <i>M. umefoliae</i> ") [Garhwal, Uttarakhand, India]
<i>M. dycei</i> Carver, 1961	?	12		Kurl and Chauhan 1986d, 1987a [Solani, Himachal Pradesh, India], Kar et al. 1990 [India]
<i>M. fataunae</i> Shinji, 1924	P(c)	8		Blackman 1986 [Japan]
<i>M. formosanus</i> Takahashi, 1923	?	12		Pal and Khuda-Bukhsh 1980 [Sonprayag, Uttarakhand, India], Khuda-Bukhsh and Pal 1986a [Garhwal, Uttarakhand, India]
<i>M. hemerocallis</i> Takahashi, 1921	?	8		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
		12		Blackman and Eastop 2006 [China; Kenya; Brazil]
<i>M. lythri</i> (Schränk, 1801)	P(c)	12		Kuznetsova and Shaposhnikov 1973 (as <i>M. (Nevskia) lythri</i> Schr.) [Crimea, Ukraine]
<i>M. mumecola</i> (Matsumura, 1917)	?	12		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Khuda-Bukhsh and Pal 1986a [Garhwal, Uttarakhand, India]
<i>M. obtusirostris</i> S.K. David, Narayanan & Rajasingh, 1971	?P(c)	12		Kurl and Chauhan 1987a [Barog, Himachal Pradesh, India]
<i>M. ornatus</i> Laing, 1932	P(o), ?P(c)	12		Blackman 1980 [Great Britain], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India], Kapoor and Gautam 1994 [Himachal Pradesh, India]
<i>M. sorbi</i> Bhattacharya & Chakrabarti ex Maity, Bhattacharya & Chakrabarti, 1982	P(c)	12		Khuda-Bukhsh and Pal 1983a [Garhwal, Uttarakhand, India]
<i>M. varians</i> Davidson, 1912	P(c)	12		Blackman 1980 [Great Britain], Bizzaro et al. 1999 [Italy]
		13		Blackman 1980 [USA]
<i>M. (Nectarosiphon) ajugae</i> Schouteden, 1903	P(c)	12		Gut 1976 [Holland]
<i>M. (N.) antirrhinii</i> (Macchiati, 1883)	P(o), ?P(c)	11, 12, 13		Hales et al. 2000 [Australia]
		11, 12, 13, 14		Wilson et al. 2003 [Great Britain; France; Canada; Australia], Blackman and Eastop 2015 [?]
		13		Blackman and Spence 1996 (clone) [Great Britain], Hales 1993 [Australia]
		13, 14		Blackman 1987b [Europe; North America], Spence et al. 1998, Terradot et al. 1999 [Great Britain]
<i>M. (N.) asteriae</i> Shinji, 1941	?	12		Blackman 1986 [Japan]
<i>M. (N.) certus</i> (Walker, 1843)	P(c), P(o)	12		Gut 1976 [Holland], Blackman 1987b [?], Spence et al. 1998 [Great Britain, USA]
<i>M. (N.) dianthicola</i> Hille Ris Lambers, 1966	P(o)	14 (heterozygous)		Blackman 1980 [Great Britain; New Zealand], Blackman 1987b [?]
<i>M. (N.) icelandicus</i> Blackman, 1986	P(c)	10		Blackman and Eastop 2015 [?]
<i>M. (N.) ligustri</i> (Mosley, 1841)	P(c)	12		Blackman 1980 [Great Britain]
<i>M. (N.) myosotidis</i> (Börner, 1950)	P(c)	12		Gut 1976 [Holland]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>M. (N.) persicae persicae</i> (Sulzer, 1776)	P(c), P(o)	8		Chattopadhyay and Raychaudhuri 1980 [Kolkata, West Bengal, India]
		8, 12, 13		Raychaudhuri and Das 1987 [India]
		10, 11, 12		Misra and Kurl 1983 [Jodhpur, Rajasthan, India]
		10, 11, 12, 13		Khuda-Bukhsh 1980 (as <i>Macrosiphum</i>) [Garhwal, Uttarakhand, India], Kurl 1986 [Meghalaya, India]
		11, 12		Hales 1993 [Australia], Spence and Blackman 2000 [clon]
		11, 12, 13		Spence et al. 1998 [France; Great Britain; lab. cultures]
		11, 12, 18 (triploid)		Yang and Zhang 2000, Yang et al. 2000 [China]
		12/11	XX/X0	Hales and Mittler 1983, 1987 [Australia], Searle and Mittler 1991 [Washington, USA]
		12		Shinji 1941b [Japan], Colling 1955 [Great Britain], Dionne and Spicer 1957 (as <i>M. solanifolii</i>), Sun and Robinson 1966, Robinson and Chen 1969a [Canada], Kuznetsova 1969 (as <i>Myzodes</i>) [Alma-Ata, Kazakhstan], Kuznetsova and Shaposhnikov 1973 (as <i>Myzodes</i>) [St. Petersburg, Russia], Kulkarni and Kacker 1980, 1981b [India], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Blackman 1986 [Japan] (based on 2n (♂) = 6 (Shinji 1941b)), Khuda-Bukhsh and Pal 1986a [Garhwal, Uttarakhand, India], Blackman 1987b [?], Kar et al. 1990 [India], Gautam and Sharma 1990 [Himachal Pradesh, India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India], Blackman and Spence 1996 [Great Britain], Spence and Blackman 1998 [Great Britain], Terradot et al. 1999 [Great Britain; France; Spain; Cuba], Wilson et al. 2002 [Australia], Gautam and Dhatwalia 2003 [Solan, Himachal Pradesh, India], Samkaria et al. 2010 [Palampur, Himachal Pradesh, India], Jangra et al. 2014 [Jammu and Kashmir, India]
		12, 13		Blackman and Takada 1976, 1977, Blackman 1986 [Japan],
		12, 14		Sethi and Nagaich 1972 [Shimla, Himachal Pradesh, India]
		12, 13, 14		Blackman 1971 (as <i>Myzodes</i>) [Great Britain; France], Blackman, Brown and Eastop 1987 [Europe; Japan; USA], Monti et al. 2012a [Italy; Great Britain], Rivi et al. 2012 [Italy]
		14		Cognetti 1961a, b (as <i>Myzodes</i>), Cognetti and Cognetti-Varriale 1961 [Italy]
		12, 13, 14, 15, 16, 17		Monti et al. 2012b [clone from Hertfordshire, Great Britain]
		12/11, 11, 12, 13, 14, 18 (triploid)	XX/X0	Blackman 1980 [Europe; Japan; USA; Chile; New Zealand]
		12		Blackman 1987b [North America], Harlow et al. 1991 [North Carolina, USA], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India], Terradot et al. 1999 [France]
<i>M. (N.) persicae nicotianae</i> Blackman, 1987	P(c), P(o)	18 (triploid)		Takada et al. 1978 [Japan]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>M. (Sciomyzus) ascalonicus</i> Doncaster, 1946	P(o)	12		Gut 1976 [Holland], Blackman 1987b [?], Kapoor and Gautam 1994 [Shimla, Himachal Pradesh, India], Blackman and Spence 1996 [Great Britain], Gautam and Dhatwalia 2003 [Solan, Himachal Pradesh, India]
<i>M. (S.) cymbalariae</i> Stroyan, 1954	P(o), ?P(c)	12		Blackman 1980 [Great Britain], Blackman 1987b [?]
<i>Myzus</i> sp. 1	?	10		Khuda-Bukhsh and Pal 1986a [Garhwal, Uttarakhand, India]
<i>Myzus</i> sp. 2	?	12		Khuda-Bukhsh and Kar 1990 [Kalimpong, West Bengal, India]
<i>Myzus</i> sp. 3	?	12		Kar et al. 1990 [India]
<i>Nasonovia compositellae nigra</i> (Hille Ris Lambers, 1931)	P(o), P(c)	11 (heterozygous)		Blackman 1980 (as <i>N. nigra</i> (Hille Ris Lambers, 1931)) [Great Britain], Blackman and Eastop 2006 [Great Britain] (one sample)
<i>N. jammuensis</i> Verma, 1966	?	12		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>N. ribisnigri</i> (Mosley, 1841)	P(c)	12		Blackman 1980 [Great Britain]
		14		Shinji 1941a (as <i>Amphorophora ribicola</i>) [Japan]
<i>N. rostrata</i> David & Hameed, 1974	P(c)	12		Pal and Khuda-Bukhsh 1980, Khuda-Bukhsh and Pal 1986b [Triyuginarayan, Uttarakhand, India]
<i>N. (Kakimia) alpina</i> (Gillette & Palmer, 1928)	?	10		Blackman and Eastop 2015 [?]
<i>N. (K.) aquilegiae</i> (Essig, 1917)	P(c)	10		Sun and Robinson 1966, Robinson and Chen 1969a (as <i>Kakimia essigi</i> (Gillette & Palmer, 1928)) [Canada]
<i>N. (K.) cymosbati</i> (Oestlund, 1887)	P(c)	10		Sun and Robinson 1966, Robinson and Chen 1969a (as <i>Kakimia</i> and as <i>Kakimia thomasi</i> (Hottes & Frison 1931)) [Canada]
<i>N. (K.) dasyphylli</i> Stroyan, 1957	P(c), P(o)	12 (heterozygous)		Blackman and Eastop 2006 [Great Britain] (one sample)
<i>Nearctaphis bakeri</i> (Cowen, 1895)	P(c), P(o)	12		Blackman 1980 [Great Britain], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>N. californica</i> Hille Ris Lambers, 1970	?P(c)	12		Blackman and Eastop 1994 [?]
<i>Neoceruraphis viburnicola</i> (Gillette, 1909)	P(c)	14		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>Neomyzus circumflexus</i> (Buckton, 1876)	P(o)	8		Blackman 1980 [Great Britain], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India], Gautam and Dhatwalia 2003 [Hamirpur, Himachal Pradesh, India]
		10		Kar and Khuda-Bukhsh 1986, Khuda-Bukhsh and Kar 1990 [Kalimpong, West Bengal, India]
<i>N. parthenocisi</i> (Takahashi, 1965)	?	12		Blackman 1986 (as <i>Aulacorthum (Neomyzus)</i>) [Japan]
<i>N. parthenocisi</i> (Takahashi, 1965)	?	12		Blackman 1986 (as <i>Aulacorthum (Neomyzus)</i>) [Japan]
<i>Neotoxoptera formosana</i> (Takahashi, 1921)	P(o)	12		Blackman and Eastop 1984 [?]
<i>N. violae</i> (Pergande, 1900)	?	12		Blackman and Eastop 1984 (as <i>Neotoxoptera oliveri</i> (Essig, 1935)) [?]
<i>Obtusicauda coweni</i> (Hunter, 1901)	P(c)	12		Blackman and Eastop 2006 [?]
<i>Oedisiphum soureni</i> A.N. Basu, 1964	?	8		Kurl and Chauhan 1986c [Barog, Himachal Pradesh, India]
<i>Ovatomyzus boraginacearum</i> Eastop, 1952	P(o)	12		Gut 1976 [Holland]
<i>O. stachyos</i> Hille Ris Lambers, 1947	?P(o)	12		Blackman and Eastop 2006 [?]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Ovatus crataegarius</i> (Walker, 1950)	P(c), P(o)	12		Shinji 1941a (as <i>Phorodon menthae</i>) [Japan], Blackman and Eastop 1984 [?], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Blackman 1986 [Japan] (based on 2n (♂) = 6 (Shinji 1941a)), Gautam and Dhatwalia 2003 [Shimla, Himachal Pradesh, India]
<i>O. insitus</i> (Walker, 1849)	P(c)	12/11	XX/X0	Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia]
<i>O. malisuctus</i> (Matsumura, 1918)	P(c)	12		Chen and Zhang 1985a (as <i>Myzus</i>) [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>Paczoskia oblecta</i> Börner, 1950	?	12		Blackman 1980 [Sweden]
<i>Paradoxaphis aristoteliae</i> Sunde, 1988	P(c)	8		Blackman and Eastop 2015 [?]
<i>Paramyzus longirostris</i> Miyazaki, 1971	?	14		Blackman 1986 [Japan]
<i>Pentalonia kalimpongensis</i> (A.N. Basu, (1967) 1968)	P(c)	12		Khuda-Bukhsh and Kar 1990 [Kalimpong, West Bengal, India]
<i>P. nigronevosa</i> Coquerel, 1859	P(o), ?P(c)	14		Blackman and Eastop 1984 [?], Panigrahy and Patnaik 1987 [Chatrapur, Odisha, India], Khuda-Bukhsh and Kar 1990 [Kalyani, West Bengal, India]
<i>Phorodon cannabis</i> Passerini, 1860	P(c)	12		Pal and Khuda-Bukhsh 1980, Khuda-Bukhsh and Pal 1986b [Tiriyuginarayan, Uttarakhand, India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>P. humuli humuli</i> (Schrank, 1801)	P(c)	12		Kuznetsova and Shaposhnikov 1973 (as <i>Ph. pruni</i> Geoffr.) [Crimea, Ukraine], Blackman 1980 [Great Britain]
<i>P. h. japonensis</i> Takahashi, 1965	P(c)	12		Shinji 1941a [Japan], Blackman and Eastop 1984 [?], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Blackman 1986 [Japan] (their own data and based on n(♂) = 6 (Shinji 1941a))
<i>P. humulifoliae</i> Tseng & Tao, 1938	?P(c)	12		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
<i>Pleotrichophorus duponti</i> Hille Ris Lambers, 1935	P(c)	14		Blackman 1980 [Great Britain]
<i>P. glandulosus</i> (Kaltenbach, 1846)	P(c)	14		Blackman 1980 [Great Britain]
<i>Plocamaphis flocculosa</i> (Weed, 1891)	P(c)	30–34?		Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia]
<i>Protaphis knowltoni</i> (Hottes & Frison, 1931)	P(c)	8		Sun and Robinson 1966, Robinson and Chen 1969a (as <i>Aphis</i>) [Canada]
<i>P. middletonii</i> (Thomas, 1879)	P(c), ?P(o)	8		Blackman 1980 (as <i>Aphis armoraciae</i> Cowen, 1895) [USA]
		8, 9		Blackman 1980 (as <i>Aphis (Protaphis) maidiradicis</i> Forbes, 1891) [USA]
<i>P. pseudocardui</i> (Theobald, 1915)	?	8		Blackman and Eastop 2015 [?]
<i>P. terricola</i> (Rondani, 1847)	P(c)	8		Blackman 1980 (as <i>Aphis (Protaphis)</i>) [Spain]
<i>Protaphis</i> sp.	?	8, 9		Blackman 1980 (as <i>Aphis (Protaphis)</i>) [Iran] (from <i>Artemisia dracuncululus</i>)
<i>Pseudocercidis rosae</i> Richards, 1961	P(c)	12		Robinson and Chen 1969a [Canada]
<i>Pseudomegoura magnoliae</i> (Essig & Kuwana, 1918)	P(o), P(c)	12		Blackman 1986 [Japan] (their own data ex. cult. on potato and based on n(♂) = 6 (Shinji 1927))
<i>Pterocomma bicolor</i> (Oestlund, 1887)	P(c)	8		Robinson and Chen 1969a [Canada]
<i>P. jacksoni</i> Theobald, 1921	?	30–34?		Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>P. konoi</i> Hori, 1939	P(c)	8		Blackman 1986 [Japan]
<i>P. pilosum</i> Buckton, 1879	P(c)	8		Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia]
<i>P. populeum</i> (Kaltenbach, 1843)	P(c)	8		Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia]
<i>P. rufipes</i> (Hartig, 1841)	P(c)	8		Kuznetsova and Shaposhnikov 1973 (as <i>P. steinheili</i> Mordvilko, 1901) [St. Petersburg, Russia]
		8, 9		Kuznetsova 1974 (as <i>steinheili</i> Mordv.) [?]
<i>P. salicis</i> (Linnaeus, 1758)	P(c)	6		Tannreuther 1907 [USA]
		30-34?		Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia]
		58		Blackman 1980 [Great Britain], Blackman and Eastop 1994 [?]
<i>P. salijaponica</i> (Shinji, 1924)	?	8/7	XX/X0	Shinji 1931 (as <i>Melanoxantherium</i>) [Japan], Blackman 1986 (as <i>Plocamaphis</i>) [Japan] (based on n(♂) = 4 (Shinji 1931))
		22		Shinji 1927, 1941a (as <i>Melanoxantherium</i>), Blackman 1986 (as <i>Plocamaphis</i>) [Japan] (based on n(♂) = 11 (Shinji 1927, 1941))
<i>P. sanguiceps</i> Richards, 1967	?	8		Blackman and Eastop 1994 [?]
<i>P. smithiae</i> (Monell, 1879)	P(c)	8		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>P. tremulae</i> Börner, 1940	?	8		Kuznetsova and Shaposhnikov 1973 [St. Petersburg, Russia]
<i>P. yezoense</i> (Hori, 1929)	P(c)	8		Blackman and Eastop 1994 [?]
<i>Rhodobium porosum</i> (Sanderson, 1900)	P(c), P(o)	14		Kar et al. 1990 [India]
<i>Rhopalomyzus (Judenkoa) loniceriae</i> (Siebold, 1839)	P(c)	12		Sun and Robinson 1966, Robinson and Chen 1969a [Canada]
<i>Rhopalosiphoninus hydrangeae</i> (Matsumura, 1918)	P(c)	12		Shinji 1941a [Japan]
<i>Rh. latsyphon</i> (Davidson, 1912)	P(o), ?P(c)	6 (+1)		Gut 1976 [Holland]
<i>Rh. tiliae</i> (Matsumura, 1918)	P(c)	12		Shinji 1941a (as <i>Rh. adenocauli</i>), 1941b (as <i>Rh. nobukii</i>), Blackman 1986 [Japan] (based on n(♂) = 6 (Shinji 1941b))
<i>Rh. (Neorhopalosiphoninus) staphyleae</i> (Koch, 1854)	P(c), P(o)	10		Blackman and Eastop 2015 [?]
<i>Rhopalosiphum cerasifoliae</i> (Fitch, 1855)	P(c)	8		Robinson and Chen 1969a [Canada]
<i>Rh. enigmae</i> Hottes & Frison, 1931	P(c)	10		Blackman and Eastop 2006 [?]
<i>Rh. maidis</i> (Fitch, 1856)	P(o), ?P(c)	8		Sun and Robinson 1966, Robinson and Chen 1969a [Canada], Mayo and Starks 1972 [USA], Kurl 1978 [Jodhpur, Rajasthan, India], Kar and Khuda-Bukhsh 1989 [Kalimpong, West Bengal, India], Dutta and Gautam 1993, Gautam and Dhatwalia 2003 [Shimla, Himachal Pradesh, India], Samkaria et al. 2010 [Palampur, Himachal Pradesh, India]
		8, 9		Blackman 1980, Hales and Cowen 1990 [Australia], Kuznetsova and Gandrabur 1991 [Fergana, Uzbekistan], De Barro 1992 [Australia]
		8,10		Chattoopadhyay et al. 1982 [India], Panigrahy and Patnaik 1991 [Chatrapur, Odisha, India]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
		8, 9, 10		Blackman et al. 1990, Blackman and Brown 1991 [USA], Jauset et al. 2000 [Catalonia, Spain]
		8, 9, 10, 11		Blackman, Brown and Eastop 1987 [Europe; North America; Iran; Israel; Australia (Tasmania)], Brown and Blackman 1988 [all continents except Antarctica] ("there is an association between karyotype and host plant, the barley-colonizing form in the northern hemisphere having 2n = 10, whereas populations on maize and sorghum have 2n = 8")
		9, 10, 11		Kuznetsova and Gandrabur 1991 [St. Petersburg, Russia]
<i>Rh. nymphaeae</i> (Linnaeus, 1761)	P(c)	8		Kuznetsova and Shaposhnikov 1973 [Tbilisi, Georgia], Blackman and Eastop 2006 [Italy]
		16		Behura and Bohidar 1978 [India] (cited after Blackman and Eastop 2015), Kurl 1978 [Meerut, Uttar Pradesh, India],
		16, 17		Kurl 1986 [Meghalaya, India]
<i>Rh. oxyacanthae</i> (Schrank, 1801)	P(c)	10		Sun and Robinson 1966, Robinson and Chen 1969a, b (as <i>Rh. fitchii</i> (Sanderson, 1902)) [Canada], Kuznetsova and Shaposhnikov 1973 (as <i>Rh. insertum</i> Walk.) [St. Petersburg, Russia], Kuznetsova et al. 1988 (as <i>Rh. insertum</i> Walk.) [St. Petersburg, Russia], Hales and Cowen 1990 (as <i>Rh. insertum</i> Walk.) [Australia]
<i>Rh. padi</i> (Linnaeus, 1758)	P(c), P(o)	8/7	XX/X0	Fox 1957 [Pennsylvania, USA]
		8		Sun and Robinson 1966, Robinson and Chen 1969a, b [Canada], Mayo and Starks 1972 [USA], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Kurl and Misra 1979 [Rajasthan, India], Kar and Khuda-Bukhsh 1989 [Kalinpong, West Bengal, India], Kuznetsova and Gandrabur 1991 [St. Petersburg, Russia], De Barro 1992 [Australia], Valenzuela et al. 2009 [Victoria, Australia], Monti et al. 2010 [Italy]
		8, 9		Hales and Cowen 1990 [Australia]
<i>Rh. padiformis</i> Richards, 1962	?P(c)	10		Blackman and Eastop 1984 [?]
<i>Rh. rufiabdominale</i> (Sasaki, 1899)	P(c), P(o)	8		Gut 1976 [Holland], Khuda-Bukhsh and Kar 1990 [Kalyani, West Bengal, India], Hales and Cowen 1990 [Australia]
<i>Rh. rufulum</i> Richards, 1960	P(c), ?P(o)	8		Gut 1976 [Holland]
<i>Rhopalosiphum</i> sp.	?	8		Bulman et al. 2004 [New Zealand]
<i>Rhopalosiphum</i> sp. ["undescribed species"]	?	9		Hales and Cowen 1990 (similar to <i>R. padi</i>) [Australia], Valenzuela et al. 2009 [Victoria, Australia]
<i>Rhopalosiphum</i> sp. ["near <i>insertum</i> "]	?	10		Valenzuela et al. 2009 [Victoria, Australia]
<i>Roepkea marchali</i> (Börner, 1931)	P(c)	12		Kuznetsova 1968 [Georgia]
<i>Sappaphis piri</i> Matsumura, 1918	P(c)	12		Kuznetsova 1968 [Vladivostok, Russia]
<i>S. sinipiricola</i> G. Zhang, 1980	?	12		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>Schizaphis graminum</i> (Rondani, 1847(1852))	P(c), P(o)	6, 8		Rubín de Celis et al. 1997 [Brazil]
		7, 8, 12		Mayo and Starks 1972 [USA]
		8		Sun and Robinson 1966, Robinson and Chen 1969a [Canada], Kuznetsova and Gandrabur 1991 [Ukraine]
		8	XX/X0	Mandrioli et al. 1999 [Modena, Italia]
<i>S. mali</i> Shaposhnikov, 1979	P(c)	8		Blackman and Eastop 2006 [?]
<i>S. piricola</i> (Matsumura, 1917)	P(c)	8		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>S. rotundiventris</i> (Signoret, 1860)	P(c), P(o)	8		Blackman and Eastop 2006 [?]
<i>S. (Paraschizaphis) acori</i> (Shinji)	P(c)	8		Blackman and Eastop 2006 [?]
<i>S. (P.) rosazevedoi</i> (Ilharco, 1961)	P(o)	8		Blackman and Eastop 2006 [?]
<i>S. (P.) scirpi</i> (Passerini, 1874)	P(c)	8		Gut 1976 (as <i>Paraschizaphis</i>) [Holland]
<i>Semiaphis heraclei</i> (Takahashi, 1921)	P(c)	8		Blackman and Eastop 1984 [?], Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015), Blackman 1986 [Japan], Gautam and Kapoor 2002 [Una, Himachal Pradesh, India]
		10		Pal and Khuda-Bukhsh 1983 [Garhwal, Uttarakhnad, India]
<i>Shinjia orientalis</i> (Mordvilko, 1929)	P(c), ?P(o)	12		Shinji 1941b (as <i>Microtarsus pterydijoliae</i>), Blackman 1986 [Japan] (based on n(♂) = 6 (Shinji 1941b)), Blackman and Eastop 2006 [?]
<i>Sinomegoura citricola</i> (van der Goot, 1917)	P(o)	12		Kulkarni 1984 [Darjeeling, West Bengal, India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
		16		Chen and Zhang 1985b (cited after Blackman and Eastop 2015)
		18		Kar and Khuda-Bukhsh 1986, Khuda-Bukhsh and Kar 1990 [Kalinpong, West Bengal, India]
<i>S. photiniae</i> (Takahashi, 1936)	?	18		Khuda-Bukhsh and Kar 1990 [Kalyani, West Bengal, India]
<i>S. pyri</i> A.K. Ghosh & D.N. Raychaudhuri, 1968	?	8		Kar et al. 1990 [India]
<i>S. rhododendri</i> (Takahashi, 1937)	?	18		Gautam and Kumar 2006 [Shimla, Himachal Pradesh, India]
<i>Sitobion alopecuri</i> (Takahashi, 1921)	P(c)	18		Blackman and Eastop 2015 [British Columbia]
<i>S. aulacorthoides</i> (David, Narayanan & Rajasingh, (1970) 1971)	?	18		Blackman and Eastop 2015 [?]
<i>S. avenae</i> (Fabricius, 1775)	P(c), P(o)	18		Sun and Robinson 1966, Robinson and Chen 1969a (as <i>Macrosiphum</i>) [Canada], Kuznetsova and Shaposhnikov 1973 [Crimea, Ukraine], Kuznetsova and Gandrabur 1991 [St.Petersburg, Russia], Rubín de Celis et al. 1997 [Brazil]
<i>S. fragariae</i> (Walker, 1848)	P(c)	18		Kuznetsova and Shaposhnikov 1973 [Crimea, Ukraine], Gautam and Kapoor 2002 [Una, Himachal Pradesh, India]
<i>S. graminis</i> Takahashi, 1950	?P(o)	18		Kurl and Chauhan 1986a (as <i>Macrosiphum</i>) [Jwalaji, Himachal Pradesh, India]
<i>S. gravelii</i> (van der Goot, 1917)	?P(c)	12		Khuda-Bukhsh and Basu 1987 (as <i>M. spinotibium</i> on <i>Artemisia vulgaris</i>) (cited after Blackman and Eastop 2015)
<i>S. ibarae</i> (Matsumura, 1917)	?P(o)	14/13		Shinji 1941a (as <i>Macrosiphum</i>) [Japan]
<i>S. indicum</i> A.N. Basu, 1964	P(o)	17, 18		Kurl 1986 (as <i>Macrosiphum</i>) [Meghalaya, India]
		18		Kurl 1980b (as <i>Macrosiphum</i>) [Meghalaya, India]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>S. luteum</i> (Buckton, 1876)	P(o)	12		Gut 1976 [Holland]
<i>S. miscanthi</i> (Takahashi, 1921)	P(o), ?P(c)	14		Kurl and Chauhan 1986a (as <i>Macrosiphum</i>) [Solan, Himachal Pradesh, India]
		18		Kurl and Chauhan 1987a (as <i>Macrosiphum</i>) [Solan, Himachal Pradesh, India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
		17, 18		Turak and Hales 1990 [Australia]
		17, 18, 20		Hales et al. 1990, Sunnucks and Hales 1996, Hales et al. 2010 [Australia]
		17, 18, 20, 21		Sunnucks et al. 1996, Hales et al. 1998 [Australia]
		17, 18, 20, 22		Wilson et al. 1999 [New Zealand]
<i>S. nigriectarium</i> (Theobald, 1915)	?	18		Blackman 1980 [Kenya]
<i>S. ochnearum</i> (Eastop, 1959)	?	18		Blackman and Eastop 2006 [?]
<i>S. pseudoluteum</i> A.K. Ghosh, 1969	?	18		Kar et al. 1990 [India]
<i>S. rosaeiformis</i> (Das, 1918)	P(c)	14, 18		Gautam and Dutta 1994 [Shimla, Himachal Pradesh, India]
		16, 17, 18		Kurl 1986 (as <i>Macrosiphum</i> (<i>Sitobion</i>)) [Meghalaya, India]
		18		Khuda-Bukhsh 1980 (as <i>Macrosiphum</i>) [Garhwal, Uttarakhand, India], Kulkarni and Kacker 1981a [Kursiong, West Bengal, India], Kurl and Misra 1983 (as <i>Macrosiphum</i> (<i>S. rosaeiformis</i>)) [Jodhpur, Rajasthan, India], Raychaudhuri and Das 1987 [India], Kar and Khuda-Bukhsh 1989 [Shillong, Meghalaya, India], Kar et al. 1990 [India]
<i>S. rosivorum</i> (G. Zhang, 1980)	?	18		Chen and Zhang 1985a (as <i>Macrosiphum</i>) [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>S. takahashii</i> (Eastop, 1959)	?	18		Khuda-Bukhsh and Kar 1989a (cited after Blackman and Eastop 2015)
<i>S. wikstroemiae</i> (Mamet, 1939)	?	16		Blackman 1980 [Kenya]
<i>Sitobion</i> sp. <i>prope avenae</i> (Fabricius, 1775)	?	12		Kapoor Gautam 1994 [Shimla, Himachal Pradesh, India]
<i>S. luteum</i> (Buckton, 1876)	P(o)	12		Gut 1976 [Holland]
<i>S. miscanthi</i> (Takahashi, 1921)	P(o), ?P(c)	14		Kurl and Chauhan 1986a (as <i>Macrosiphum</i>) [Solan, Himachal Pradesh, India]
		18		Kurl and Chauhan 1987a (as <i>Macrosiphum</i>) [Solan, Himachal Pradesh, India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
		17, 18		Turak and Hales 1990 [Australia]
		17, 18, 20		Hales et al. 1990, Sunnucks and Hales 1996, Hales et al. 2010 [Australia]
		17, 18, 20, 21		Sunnucks et al. 1996, Hales et al. 1998 [Australia]
		17, 18, 20, 22		Wilson et al. 1999 [New Zealand]
<i>S. nigriectarium</i> (Theobald, 1915)	?	18		Blackman 1980 [Kenya]
<i>S. ochnearum</i> (Eastop, 1959)	?	18		Blackman and Eastop 2006 [?]
<i>S. pseudoluteum</i> A.K. Ghosh, 1969	?	18		Kar et al. 1990 [India]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>S. rosaeiformis</i> (Das, 1918)	P(c)	14, 18		Gautam and Dutta 1994 [Shimla, Himachal Pradesh, India]
		16, 17, 18		Kurl 1986 (as <i>Macrosiphum</i> (<i>Sitobion</i>)) [Meghalaya, India]
		18		Khuda-Bukhsh 1980 (as <i>Macrosiphum</i>) [Garhwal, Uttarakhand, India], Kulkarni and Kacker 1981a [Kursiong, West Bengal, India], Kurl and Misra 1983 (as <i>Macrosiphum</i> (<i>S.</i>) <i>rosaeiformis</i>) [Jodhpur, Rajasthan, India], Raychaudhuri and Das 1987 [India], Kar and Khuda-Bukhsh 1989 [Shillong, Meghalaya, India], Kar et al. 1990 [India]
<i>S. rosivorum</i> (G. Zhang, 1980)	?	18		Chen and Zhang 1985a (as <i>Macrosiphum</i>) [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>S. takahashii</i> (Eastop, 1959)	?	18		Khuda-Bukhsh and Kar 1989a (cited after Blackman and Eastop 2015)
<i>S. wikstroemiae</i> (Mamet, 1939)	?	16		Blackman 1980 [Kenya]
<i>Sitobion</i> sp. prope <i>avenae</i> (Fabricius, 1775)	?	12		Kapoor and Gautam 1994 [Shimla, Himachal Pradesh, India]
<i>Sitobion</i> sp. prope <i>fragariae</i> (Walker, 1848)	?	18		Turak and Hales 1990, Hales et al. 1990, De Barro 1992, Sunnucks et al. 1996, Hales et al. 1998 [Australia], Wilson et al. 1999 [New Zealand]
<i>Sitobion</i> sp. prope <i>rosaeiformis</i> (Das, 1918)	?	18		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>Sitobion</i> sp.	?	12		Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>Sorbaphis chaetosiphon</i> Shaposhnikov, 1950	P(c)	38		Blackman 1986 [Japan]
<i>Staticobium limonii</i> (Contarini, 1847)	?	12		Blackman and Eastop 2006 [?]
<i>Titanosiphon neoartemisiae</i> (Takahashi, 1921)	P(c), P(o)	8		Blackman and Eastop 2006 [for specimens on <i>A. dracunculus</i> in Iran]
<i>Tricaudatus polygوني</i> (Narzikulov, 1953)	P(c)	8		Kar et al. 1990 [India]
<i>Trichosiphonaphis</i> (<i>Xenomyzus</i>) <i>polygوني</i> (van der Goot, 1917)	?	12		Chen and Zhang 1985a (as <i>Trichosiphonaphis ishimikawae</i> (Shinji 1941)) (cited after Blackman and Eastop 2015)
<i>T. (X.) polygonifoliae</i> (Shinji, 1944)	P(c)	12		Blackman and Eastop 2015 [?]
<i>T. (X.) tade</i> (Shinji, 1927)	?	10/9	XX/X0	Shinji 1927, 1931, 1941a (as <i>Carolinaia</i>), Blackman 1986 [Japan] (based on n(♂) = 5 (Shinji 1927, 1931))
		12		Blackman 1986 [Japan]
<i>Tubaphis clematophila</i> (Takahashi, 1965)	?	12		Blackman 1986 [Japan]
<i>Tuberocephalus</i> (<i>Trichosiphoniella</i>) <i>higansakurae</i> (Monzen, 1927)	P(c)	12		Blackman 1986 [Japan]
<i>T. (T.) liaoningensis</i> G. Zhang & Zhong, 1976	P(c)	12		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>T. (T.) misakurae</i> Moritsu & Hamasaki, 1983	P(c)	12		Blackman and Eastop 1994 [?]
<i>T. (T.) momonis</i> (Matsumura, 1917)	P(c)	12		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>Uroleucon achilleae</i> (Koch, 1855)	P(c)	12		Blackman 1980 [Great Britain]
<i>U. ambrosiae</i> (Thomas, 1878)	P(c), P(o)	12		Olive 1967 (as <i>Dactynotus</i>) [USA], Robinson and Chen 1969a (as <i>Dactynotus</i>) [Canada]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>U. chondrillae</i> (Nevsky, 1929)	P(c)	12		Blackman and Eastop 2006 [?]
<i>U. chrysopsidicola</i> (Olive, 1963)	P(c)	12		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. cirsii</i> (Linnaeus, 1758)	P(c)	10		Sun and Robinson 1966, Robinson and Chen 1969a (as <i>Dactynotus</i>) [Canada], Kuznetsova and Gandrabur 1991 [St.Petersburg, Russia]
<i>U. formosanum</i> (Takahashi, 1921)	?	12	XX/X0	Shinji 1927, 1931 (as <i>Macrosiphum sonchi</i>), Blackman 1986 [Japan] (based on n(♂) = 6 (Shinji 1927, 1931))
<i>U. fuscaudatum</i> Chakrabarti & D.N. Raychaudhuri, 1978	?	12		Pal and Khuda-Bukhsh 1980, Khuda-Bukhsh and Pal 1986b [Rambara, Uttarakhand, India]
<i>U. cichorii grossum</i> (Hille Ris Lambers, 1939)	P(c)	12		Gut 1976 [Holland]
<i>U. hypochoeridis</i> (Fabricius, 1779)	P(c)	12		Gut 1976 [Holland]
<i>U. jaceicola</i> (Hille Ris Lambers, 1939)	P(c)	12		Gut 1976 [Holland]
<i>U. longisetosum</i> Chakrabarti & Verma, 1975	P(c)	10		Pal and Khuda-Bukhsh 1980, Khuda-Bukhsh and Pal 1986b [Gobindoghat, Uttarakhand, India]
<i>U. macolai</i> (Blanchard, 1932)	P(c)	12		Blackman and Eastop 2006 [?]
<i>U. nigrotuberculatum</i> (Olive, 1963)	P(c)	12		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. paucosensoriatum</i> (Hille Ris Lambers, 1960)	P(c)	12		Robinson and Chen 1969a (as <i>Dactynotus</i>) [Canada]
<i>U. pseudambrosiae</i> (Olive, 1963)	?	12		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. pseudotanacetii</i> (Verma, 1969 (1970))	P(c)	12		Kurl and Chauhan 1986a, 1987a [Kangra, Himachal Pradesh, India]
<i>U. reynoldense</i> (Olive, 1965)	?	12		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. rudbeckiae</i> (Fitch, 1851)	P(c)	12		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. russellae</i> (Hille Ris Lambers, 1960)	P(c)	12		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. simlaense</i> Chakrabarti, A.K. Ghosh & D.N. Raychaudhuri, 1971	?	12		Kurl and Chauhan 1987a [Kandaghat, Himachal Pradesh, India]
<i>U. sonchellum</i> (Monell, 1879)	P(c)	12		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. sonchi</i> (Linnaeus, 1767)		12	XX/X0	Olive 1967 (as <i>Dactynotus</i>) [USA]
	P(c), ?P(o)	12		Kulkarni and Kacker 1981a [Dadhau, Himachal Pradesh, India], Kurl and Chauhan 1986c, 1987a [Barog, Himachal Pradesh, India], Kar et al. 1990 [India], Dutta and Gautam 1993 [Shimla, Himachal Pradesh, India]
<i>U. tanacetii</i> (Linnaeus, 1758)	P(c)	12		Gut 1976 [Holland]
<i>U. tussilaginis</i> (Walker, 1850)	P(c)	8?		Kuznetsova 1974 (as <i>Dactynotus basalis</i> Walker?) [?] (Blackman and Eastop 2015 supposed that the karyotype in Kuznetsova 1974 illustrated resembles that of <i>Acyrtosiphon pisum</i>)
<i>U. (Belochilum) inulae</i> (Ferrari, 1872)	?	12		Blackman and Eastop 2006 [?]
<i>U. (Lambersius) anomalae</i> (Hottes & Frison, 1931)	?	12		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. (L.) bradburyi</i> (Olive, 1965)	P(c)	12		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. (L.) erigeronense</i> (Thomas, 1878)	P(c)	12		Blackman and Eastop 2015 [?]
<i>U. (L.) gravicorne</i> (Patch, 1919)	P(c)	12		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. (L.) luteolum</i> (Williams, 1911)	P(c)	12		Olive 1967 (as <i>Dactynotus tissoti</i> Boudreaux, 1948 (1949)) [USA]
<i>U. (L.) penderum</i> Robinson, 1986	?	12		Blackman and Eastop 2006 [British Columbia, Canada]

Taxon	Life cycle	2n ♀/♂	Genetic system, ♀/♂	References and collecting data
<i>U. (L.) richardsi</i> Robinson, 1964	P(c)	12		Robinson and Chen 1969a (as <i>Dactynotus</i>) [Canada]
<i>U. (Uromelan) carthami</i> (Hille Ris Lambers, 1948)	?	12		Blackman and Eastop 2006 [?]
		14		Khuda-Bukhsh and Kar 1990 [Kalyani, West Bengal, India]
<i>U. (U.) compositae</i> (Theobald, 1915)	P(o)	12		Kurl and Misra 1983 (as <i>Dactynotus</i>) [Jodhpur, Rajasthan, India], Khuda-Bukhsh and Kar 1990 [Shillong, Meghalaya, India]
<i>U. (U.) gobonis</i> (Matsumura, 1917)	P(c), P(o)	12		Blackman and Eastop 1984, Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
		14	XX/X0	Shinji 1927, 1931, 1941a (as <i>Macrosiphum</i>), Blackman 1986 [Japan] (based on n(♂) = 7 (Shinji 1931))
<i>U. (U.) helianthicola</i> (Olive, 1963)	?	12		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. (U.) himachali</i> L.K. Ghosh, 1975	?	14		Kar et al. 1990 [India]
<i>U. (U.) illini</i> (Hottes & Frison, 1931)	P(c)	12		Blackman and Eastop 2006 [?]
<i>U. (U.) jaceae</i> (Linnaeus, 1758)	P(c)	12		Blackman 1980 [Great Britain], Kar et al. 1990 [India]
<i>U. (U.) rurale</i> (Hottes & Frison, 1931)	P(c)	10		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. (U.) taraxaci</i> (Kaltenbach, 1843)	P(c)	12		Sun and Robinson 1966 (as <i>Dactynotus</i>), Robinson and Chen 1969a (as <i>Dactynotus</i>) [Canada]
<i>U. (U.) tuataiae</i> Olive, 1963	?	12		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>U. (U.) verbesinae</i> (Boudreaux, 1949)	?	10		Olive 1967 (as <i>Dactynotus</i>) [USA]
<i>Uroleucon</i> sp. 1	?	12		Robinson and Chen 1969a (as <i>Dactynotus</i>) [Canada] (five different taxonomic forms)
<i>Uroleucon</i> sp. 2	?	12		Chen and Zhang 1985a [Beijing area, China] (cited after Blackman and Eastop 2015)
<i>Utamphorophora crataegi</i> (Monell, 1879)	P(c)	10		Robinson and Chen 1969a [Canada]
<i>U. humboldti</i> (Essig, 1941)	P(c), P(o)	20		Robinson and Chen 1969a (as <i>Myzodes physocarpi</i> Pepper, 1950) [Canada], Blackman 1980 [Great Britain]
<i>Vesiculaphis caerulea</i> Miyazaki, 1980	?	6		Blackman 1986 [Japan]
<i>V. cephalata</i> Miyazaki, 1971	P(c)	20		Blackman 1986 [Japan]
<i>V. theobaldi</i> Takahashi, 1930	P(c), P(o)	36		Blackman and Eastop 2006 [Great Britain, ?anholocyclic populations]
		38		Blackman and Eastop 2006 [Great Britain, ?anholocyclic populations]
		40		Gut 1976 [Holland]
<i>Vesiculaphis</i> sp.	?	24		Gautam and Kumar 2006 [Shimla, Himachal Pradesh, India]
<i>Wahlgreniella nervata</i> (Gillette, 1908)	?P(c), P(o)	12		Blackman 1980 [Great Britain]
<i>W. vaccinii</i> (Theobald, 1924)	P(c)	12		Blackman and Eastop 2015 [?]
<i>Xerobion cinae</i> (Nevsky, 1928)	P(c)	8		Blackman 1980 [Iran]

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Molecular cytogenetic studies in the ladybird beetle *Henosepilachna argus* Geoffroy, 1762 (Coleoptera, Coccinellidae, Epilachninae)

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Abstract

The ladybird *Henosepilachna argus* Geoffroy, 1762 has been cytogenetically studied. In addition we have conducted a review of chromosome numbers and the chromosomal system of sex determination available in the literature in species belonging to the genus *Henosepilachna* and in its closely related genus *Epilachna*. Chromosome number of *H. argus* was $2n=18$, including the sex chromosome pair, a common diploid chromosome number within the tribe Epilachnini. The study of prophase I meiotic chromosomes showed the typical Xy_p “parachute” bivalent as in the majority of species of Coccinellidae. C-banding and fluorescent staining with AT-specific DAPI fluorochrome dye have been carried out for the first time in *H. argus*. C-banding technique revealed that heterochromatic blocks are pericentromerically located and DAPI staining showed that this heterochromatin is AT rich.

Fluorescence in situ hybridizations using rDNA and the telomeric TTAGG sequence as probes have been carried out. FISH using rDNA showed that the nucleolar organizing region is located on the short arm of the X chromosome. FISH with the telomeric sequence revealed that in this species telomeres of chromosomes are composed of the pentanucleotide TTAGG repeats. This is the first study on the telomeric sequences in Coccinellidae.

Keywords

Henosepilachna argus, karyotype, C-banding, DAPI staining, NOR, telomeres

Introduction

Insects are one of the most diverse and biggest numerous groups of metazoans. This group contains almost one million of species, half a million of which are phytophagous. Most of those phytophagous insects are considered specialist feeding on one or few plant species (Schoonhoven et al. 2005). *Henosepilachna argus* Geoffroy, 1762 (Coleoptera, Coccinellidae) the bryony ladybird is a phytophagous insect (both larvae and adults) which feeds on leaves of white bryony and other Cucurbitaceous plants, including melon or watermelon crops.

The tribe Epilachnini is included in the Epilachninae subfamily (Coccinellidae, Epilachninae) (Jadwiszczak and Wegrzynowicz 2003). Within Epilachnini, one of the most problematic questions is the distinctiveness of the genera *Epilachna* and *Henosepilachna*. Although both genera have been distinguished by morphological characters (Li 1993), that question is not fully elucidated. Recently Katoh et al. (2014) by using a combined dataset of NADH dehydrogenase subunit 2 (ND2) and the 28S rDNA reconstructed the phylogeny of 46 species of Epilachnini, including 16 species of *Epilachna* and 24 species of *Henosepilachna*. The results obtained by Katoh et al. (2014) suggest that *Henosepilachna* and *Epilachna* are polyphyletic but also the existence of some well-supported clades, such as Asian *Epilachna*, American *Epilachna* and Asian and Australian *Henosepilachna*. Despite this, Katoh et al. (2014) recommended that a new phylogenetic analysis has to be done, with special careful attention to both morphological and molecular analyses with a broad taxonomic representation. Thereby the taxonomy of the species belonging to the genus *Henosepilachna* remains unclear with misidentification for some species. Sometimes it is due to the existence of intraspecific variation which causes a wide variation in the external appearance and morphological characters presented by the species of this group (Naz et al. 2012).

In this paper a karyotype analysis, C-banding and fluorescent staining with the AT-specific DAPI fluorochrome dye have been carried out for the first time in *Henosepilachna argus*. In addition we have conducted a review of chromosome numbers and the chromosomal system of sex determination available in the literature in species belonging to the genera *Henosepilachna* and *Epilachna*. Fluorescence in situ hybridizations using rDNA and (TTAGG)_n as probes have also been carried out for the first time in Epilachninae. This molecular cytogenetic study could be helpful in the future for solving the problem of distinctiveness of both genera.

Material and methods

Chromosome preparations, C-banding and DAPI staining

Chromosome spreads were obtained from adult male gonads according to the technique described by Lorite et al. (1996a). C-banding was performed as described by

Sumner (1972) with some modifications (Palomeque et al. 2005). Staining of the chromosomes with 4'-6-diamino-2-phenyl-indol (DAPI) was performed according to Schweizer (1980).

Fluorescence *in situ* hybridization

The physical mapping of 18S and 28S rDNA loci was carried out by fluorescence *in situ* hybridization (FISH). FISH was performed as described previously (Lorite et al. 2002a, Palomeque et al. 2005). A plasmid containing the *Drosophila melanogaster* Meigen, 1830 rDNA gene (pDmr.a 51#1) (Endow 1982) was used as probe. The telomeric DNA probe was generated by polymerase chain reaction (PCR) using the (TTAGG)₆ and (TAACC)₆ oligonucleotides as primers (Lorite et al. 2002b). Both probes were labeled with biotin-16-dUTP using the biotin labeling kit from Roche (final concentration of 2 ng/ml, 50% formamide). Fluorescence immunological detection was performed using the avidin-FITC/anti-avidin-biotin system with two rounds of amplification for the rDNA probe and four rounds of amplification for the telomeric probe. Slides were counterstained with propidium iodide and DAPI.

Results and discussion

Henosepilachna argus showed 8 pairs of autosomes and the sex chromosomes X and Y. The karyotype was composed of 6 pairs of metacentric (pairs 1, 2, 3, 5, 6 and 7) and 2 pairs of submetacentric autosomes (pairs 4 and 8). The chromosome X was submetacentric and the chromosome Y was minute and seems to be acrocentric (Figure 1A and C). We have conducted a review of chromosome numbers and chromosomal system of sex determination available in the literature in species belonging to the genera *Henosepilachna* and *Epilachna* (Table 1). A variable chromosome number was given for *Henosepilachna dodecastigma* Wiedemann, 1934 with a chromosome number ranging from 6 pairs of autosomes and the sex chromosome pair to 9 pairs of autosomes and the sex chromosome pair (review in Smith and Virkki 1978). However the results showed by Saha (1973) suggest that the $2n = 14$ is the most probable chromosome number for this species. According to Sloggett and Honěk (2012) the most common diploid chromosome number within Epilachnini was 18-20 (including the sex chromosome pair), as happens in *Henosepilachna argus* with a chromosome number of $2n=18$.

Differential chromosomal staining is able to show some specific patterns helpful to distinguish chromosomes with the same size. C-banding reveals the constitutive heterochromatin (Sumner 1972). In Coccinellidae beetles the heterochromatin is associated with pericentromeric regions and short arms of chromosomes (Drets et al. 1983, Maffei et al. 2004, Rozek and Holecova 2002, among others). The application of C-banding techniques in *Henosepilachna argus* showed large heterochromatic pericentromeric blocks on all chromosomes. The small chromosome Y was almost entirely

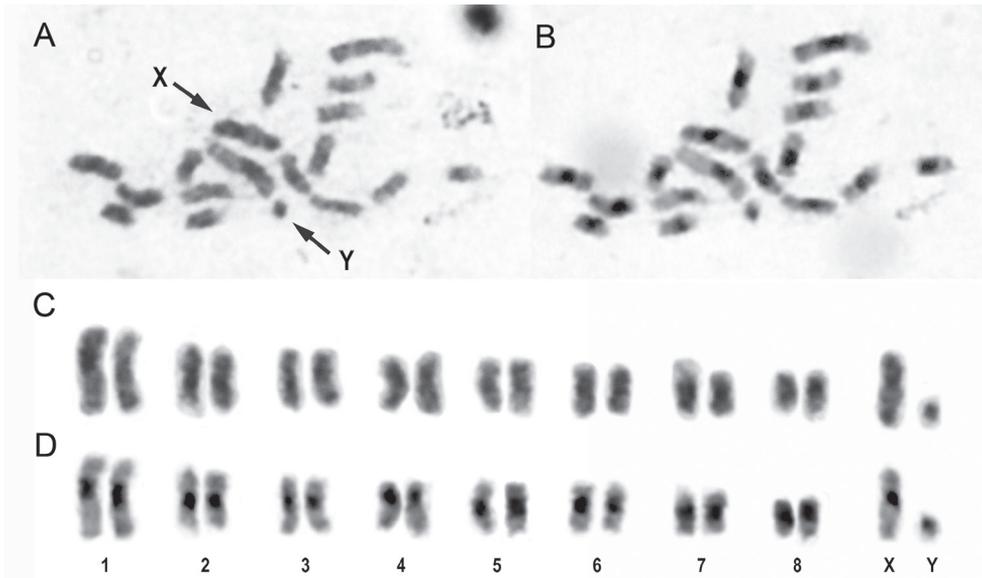


Figure 1. Metaphase plate and karyotype of *Henosepilachna argus* male. Giemsa staining (**A, C**) and C-banding (**B, D**). The arrows indicate the sex chromosomes (X and y).

Table 1. Known chromosome numbers and chromosomal system of sex determination in species belonging to the genera *Epilachna* and *Henosepilachna*.

<i>Epilachna</i>		
	2n	References
<i>Epilachna admirabilis</i> Crotch, 1874	18 Xy	Tanaka and Sasaji (1992)
<i>Epilachna borealis</i> Fabricius, 1775	18 Xy	Stevens (1906), Hoy (1918)
<i>Epilachna cacica</i> Guérin-Meneville, 1844	20 Xy	Vidal (1984)
<i>Epilachna dumerili</i> Mulsant, 1850	16 Xy	Yadav et al. (1991)
<i>Epilachna obscurella</i> Mulsant, 1850	18 Xy	Gomez and Castorena (1972)
<i>Epilachna paenulata</i> Germar, 1824	18 Xy	Drets et al. (1983)
<i>Epilachna varivestris</i> Mulsant, 1850	20 Xy	Gomez and Castorena (1972)
<i>Henosepilachna</i>		
<i>Henosepilachna chrysolina</i> Fabricius, 1775 × <i>capensis</i> Thunberg, 1784	18	Strasburger (1936)
<i>Henosepilachna dodecastigma</i> Wiedemann, 1823	20 Xy	Lahiri and Manna (1969)
	12–14	Saha and Manna (1971)
	14 Xy	Saha (1973)
<i>Henosepilachna niponica</i> Lewis, 1896	20 Xy	Yosida (1944), Tanaka and Sasaji (1992), Tsurusaki et al. (1993)
<i>Henosepilachna orientalis</i> Zimmerman, 1936	18 XY	Agarwal (1961)
<i>Henosepilachna pustulosa</i> Kono 1937	20 Xy	Yosida (1948), Tsurusaki et al. (1993)
<i>Henosepilachna septima</i> Dieke, 1947	20 Xy	Kacker (1973)
<i>Henosepilachna vigintioctomaculata</i> Motschulsky, 1857	20 Xy	Yosida (1948), Takenouchi (1955), Tsurusaki et al. (1993)
<i>Henosepilachna vigintioctopunctata</i> Fabricius, 1775	18 Xy; XY	Bose (1948), Yosida (1948), Agarwal (1961), Yadav and Pillai (1979), Tanaka and Sasaji (1992), Kobayashi et al. (2000)
<i>Henosepilachna yasutomii</i> Katakura, 1981	20 Xy	Tsurusaki et al. (1993)

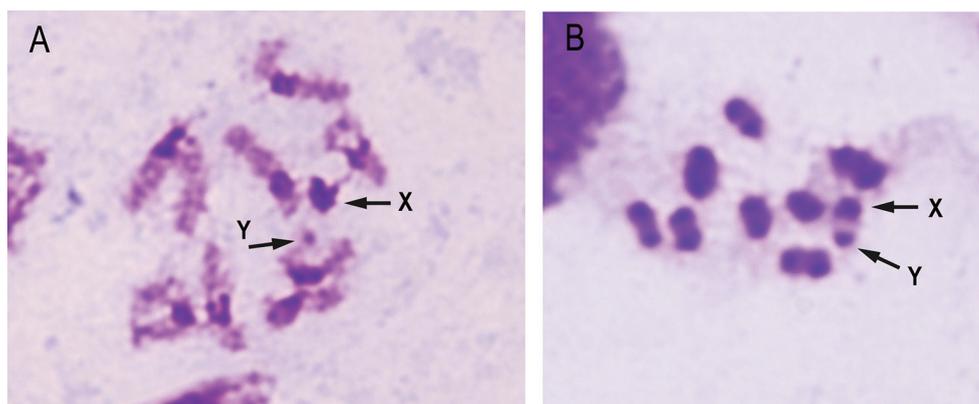


Figure 2. Giemsa staining of meiotic chromosomes at late pachytene (**A**) and in metaphase I (**B**). The arrows indicate the sex chromosomes (X and y).

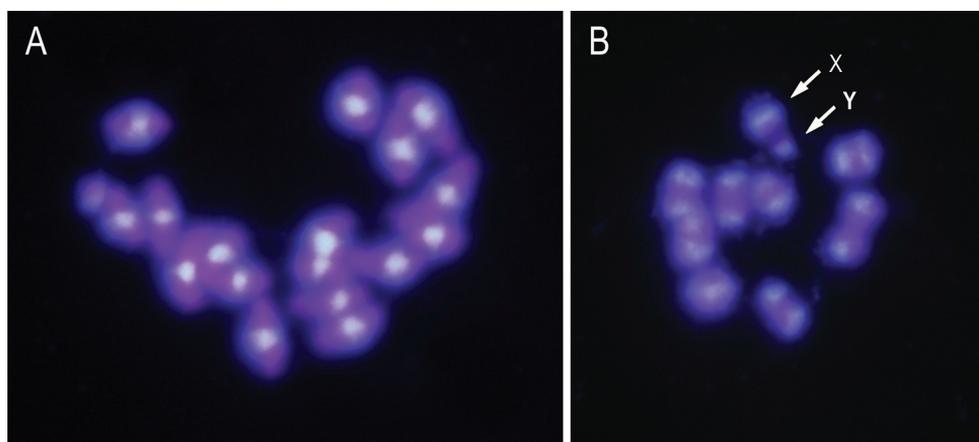


Figure 3. DAPI staining of mitotic metaphase (**A**) and meiotic metaphase I (**B**). The arrows indicate the sex chromosomes (X and y).

heterochromatic (Figure 1B and D). Similar results have been reported for other *Epilachnini* although only four species have been analyzed by C-banding methods (Drets et al. 1983, Tsurusaki et al. 1993).

The sex chromosomal system found in *Henosepilachna argus* is XX/Xy_p (Figure 2). The y chromosome was minute and for this reason it is often written with lowercase letter. When X and y chromosomes were paired in meiosis, they resemble a “parachute”. The Xy_p parachute system is considered the most common form of sex determination system in *Coccinellidae* family as well as in other families of *Coleoptera* insects (Smith and Virkki 1978, De Julio et al. 2010). However in some groups the Y chromosome has disappeared and the chromosomal system of sex determination changes to the X0 system (Angus et al. 2015).

Association of heterochromatic segments of all chromosome complement during early meiotic stages forming a single chromocenter has been described in *Epilachna*

vigintioctopunctata Fabricius, 1775 (Bose 1948) and in *Epilachna paenulata* Germar, 1824 (Drets et al. 1983). This association has not been found in *Henosepilachna argus* (Figure 2) or any other species of Epilachnini, with the exception of the two previously commented species. However, associations of heterochromatic segments of non-homologous chromosomes in chromocenters have been described in others insects such as in Triatominae (Hemiptera) (Pita et al. 2014).

DAPI staining of mitotic chromosomes displayed similar results that C-banding technique with the pericentromeric chromosome regions intensely stained (Figure 3). Equally in meiotic metaphase I bivalents the pericentromeric regions were intensely stained. DAPI staining coincident with C-banding heterochromatic pericentromeric regions has also been found in some Coleoptera, suggesting that these heterochromatic regions are rich in AT base pairs although in other Coleoptera the DAPI staining of chromosomes did not reveal any positive signal (Karagyan et al. 2012, Da Silva et al. 2015 and references therein). This varied and different banding pattern could be due to the structure and composition of insect heterochromatin, especially in relation to the heterochromatin associated proteins (Lorite et al. 1996b).

The FISH technique using rDNA showed a positive hybridization signal on the short arm of X chromosome (Figure 4). The localization of the nucleolar organizer region (NOR) is unknown in other species of Epilachnini. At this moment the chromosome location of the NOR in Coccinellidae is only known in two species, *Olla v-nigrum* Mulsant, 1866 and *Cycloneda sanguinea* Linnaeus, 1763 (Maffei et al. 2001, 2004) by Ag-NOR banding and FISH. The NOR location in both species is variable. In the first one the NOR region appears on the sex chromosomes (Maffei et al. 2001), nevertheless in the second one the NOR region appears on one pair of autosomes (Maffei et al. 2004). The variable location of the NORs has been observed in other Coleopteran families as Carabidae, Melolonthidae, Tenebrionidae or Scarabaeidae that show that rDNA sequences are located on the sex chromosomes, autosomes or both depending on the species (Oliveira et al. 2012, Arcanjo et al. 2013).

FISH showed that the TTAGG motif is present in the telomeres of the chromosomes of *H. argus* (Figure 5). The pentanucleic repetition TTAGG is considered the most common telomeric sequence in insects (Frydrychová et al. 2004). In spite of this, the DNA composition of coleopteran telomeres is very variable. Frydrychová and Marec (2002) studied the occurrence of (TTAGG)_n repeats in the telomeres of 12 species of beetles, which represent the major lineages of the phylogenetic tree of the Coleoptera order. Furthermore, the presence or absence of (TTAGG)_n repeats was independent of the phylogenetic relationships. For example, in the suborder Polyphaga six species showed positive results to TTAGG probe and three negative results. In addition in *Tribolium castaneum* Herbst, 1797 (Tenebrionidae) there has been a replacement from TTAGG repetition to TCAGG repetition (Richards et al. 2008). Mravinac et al. (2011) showed that the motif TCAGG is found in all the 19 examined species of three beetle families belonging to the superfamily Tenebrionoidea, whereas TTAGG the canonical telomeric motif of insects, is found in most but not in all of the remaining species covering four families, Cucujidae, Cerambycidae, Chrysomelidae and

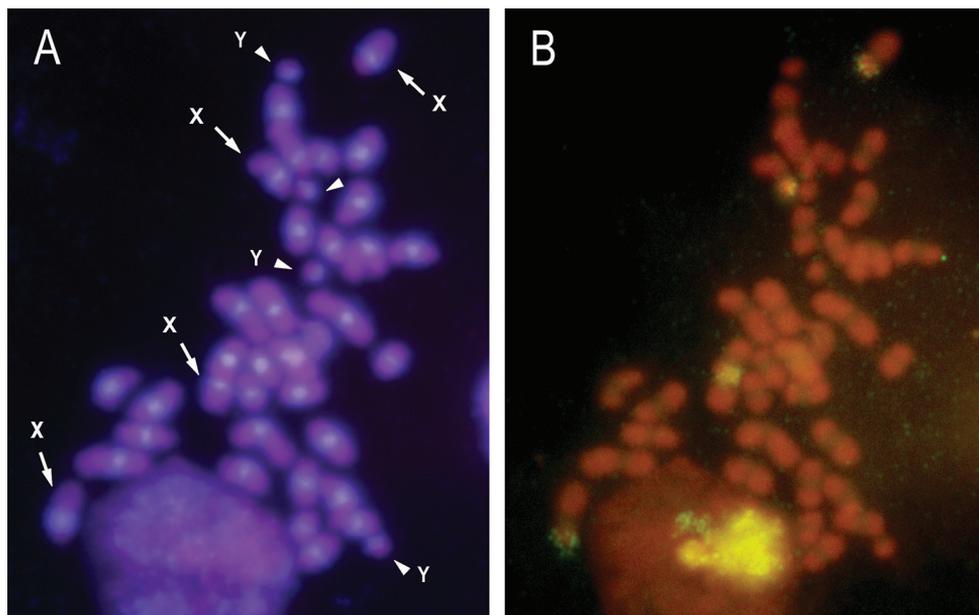


Figure 4. (A) Four mitotic metaphase plates stained with DAPI, the X chromosomes were showed by arrows, and y chromosomes were showed by the arrowhead. (B) FISH using rDNA as probe. Positive hybridization signals on the short arm of X chromosomes were showed.

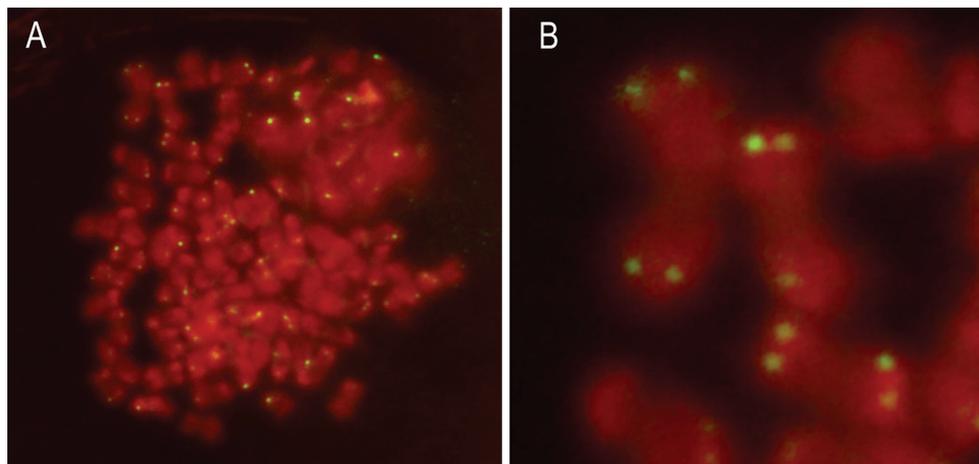


Figure 5. FISH using (TTAGG)_n as probe on mitotic chromosomes (A) and selected chromosomes (B).

Curculionidae. The analysis of the genome of *T. castaneum* also showed that multiple telomeres are formed by TCAGG repetitions interrupted by full-length and truncated non-LTR (Long Terminal Repeats) retrotransposons. The authors also suggested that this type of telomeres should be a “middle” stage between the typical telomeres like in *Apis mellifera* Linnaeus, 1761 (Hymenoptera) (Robertson and Gordon 2006) and

telomeres which are exclusively formed by non-LTR as in *Drosophila* (Diptera) (review by Zhang and Rong 2012, among others).

Thus, in this paper classical and molecular cytogenetic techniques have been performed on chromosomes of *Henosepilachna argus*. This is the first study on rDNA localization in Epilachninae. Besides, it is the first study of telomeric sequences in Coccinellidae family. This molecular cytogenetic study, in addition to expanding the knowledge of this species, could be helpful in the future for solving of the problem of distinctiveness between both genera

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Spreading of heterochromatin and karyotype differentiation in two *Tropidacris* Scudder, 1869 species (Orthoptera, Romaleidae)

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Abstract

Tropidacris Scudder, 1869 is a genus widely distributed throughout the Neotropical region where speciation was probably promoted by forest reduction during the glacial and interglacial periods. There are no cytogenetic studies of *Tropidacris*, and information allowing inference or confirmation of the evolutionary events involved in speciation within the group is insufficient. In this paper, we used cytogenetic markers in two species, *T. collaris* (Stoll, 1813) and *T. cristata grandis* (Thunberg, 1824), collected in different Brazilian biomes. Both species exhibited $2n=24,XX$ for females and $2n=23,X0$ for males. All chromosomes were acrocentric. There were some differences in the karyotype macrostructure, e.g. in the chromosome size. A wide interspecific variation in the chromosome banding (C-banding and CMA_3 /DAPI staining) indicated strong differences in the distribution of repetitive DNA sequences. Specifically, *T. cristata grandis* had a higher number of bands in relation to *T. collaris*. FISH with 18S rDNA revealed two markings coinciding with the NORs in both species. However, two analyzed samples of *T. collaris* revealed a heterozygous condition for the rDNA site of S_{10} pair. In *T. collaris*, the histone H3 genes were distributed on three chromosome pairs, whereas in *T. cristata grandis*, these genes were observed on 14 autosomes and on

the X chromosome, always in terminal regions. Our results demonstrate that, although the chromosome number and morphology are conserved in the genus, *T. cristata grandis* substantially differs from *T. collaris* in terms of the distribution of repetitive sequences. The devastation and fragmentation of the Brazilian rainforest may have led to isolation between these species, and the spreading of these repetitive sequences could contribute to speciation within the genus.

Keywords

Chromosome banding, repetitive DNA, speciation, histone H3 gene, 18S rDNA

Introduction

The genus *Tropidacris* Scudder, 1869 comprises the largest grasshoppers of the order Orthoptera, reaching up to 14 centimeters in length (Diniz-Filho et al. 2010). They have a strong influence on the food chain as prey and predators, contributing to the natural balance of the populations (Nunes 1996). These animals are of substantial ecological importance, as they are forest defoliators that feed on leaves, decaying organic matter and mosses (Amédégnato 1977). In addition, some species can be important agricultural pests that cause extreme economic losses (Poderoso et al. 2013).

Tropidacris is widely spread throughout the Neotropical region. Its natural habitats extensively vary from dense rainforests to very open areas with a dry climate (Eades et al. 2010, Diniz-Filho et al. 2010). In a review, Carbonell (1986) classified the genus into three species: *T. collaris* (Stoll, 1813), *T. descampsi* (Carbonell, 1986) and *T. cristata* (Linnaeus, 1758). The latter was subdivided into three subspecies: *Tropidacris cristata dux* (Drury, 1770), *Tropidacris cristata cristata* (Linnaeus, 1758) and *Tropidacris cristata grandis* (Thunberg, 1824) (Diniz-Filho et al. 2010). All these species have different geographical distribution. *T. collaris* has a wider distribution than *T. cristata*, while the species *T. descampsi* was described based on a single specimen from Colombia (Diniz-Filho et al. 2010).

Considering the geographical distribution of the above-mentioned taxa, Carbonell (1986) proposed that their common ancestor may have arisen in the Guiano-Amazon region. He also substantiated that speciation probably began by the geographic isolation of two populations which later developed into *T. collaris* and *T. cristata*. These events probably relate to episodes of forest reduction during the glacial and interglacial retreat periods, which caused the fragmentation of this environment and its further expansion. Barriers between the allopatric populations can be created by geological forces, favoring both genetic differentiation and speciation (Nosil 2008).

In different organisms, speciation is related to important chromosomal rearrangements. Translocations, inversions, duplications and deletions can lead to chromosome segregation problems, causing different degrees of sterility. Karyotype changes, such as amplification or dispersion of repetitive DNA sequences may also have an important role in this process. These structural rearrangements lead to reproductive barriers and thus to the formation of new biological species (Livingstone and Rieseberg 2004). For this reason, reproductive barriers are extremely important in terms of evolutionary

process (Southcott et al. 2013). Isolated genomes can accumulate differences until formation of a complete reproductive barrier (Rieseberg 2001, Noor et al. 2001, Livingstone and Rieseberg 2004).

There are no specific cytogenetic studies of *Tropidacris*, and information allowing inference or confirmation of the evolutionary events involved in speciation in the group is insufficient. Thus, we used cytogenetic markers to analyze *T. collaris* and *T. cristata grandis*, two members of the genus collected in different biomes of northeastern and southern Brazil. We intend to propose a mechanism that explains both the chromosome evolution and reproductive isolation between these taxa.

Methods

Samples and collection sites

The specimens of *T. collaris* and *T. cristata grandis* were collected from two regions of Brazil (Table 1 and Fig. 1). Individuals of both species were identified and deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP).

Chromosome preparation and conventional staining

The samples were anesthetized and dissected before fixing their testes and gastric caeca in methanol: acetic acid 3:1. The females were injected with 0.1% colchicine 6h prior to dissection. For mitotic and meiotic analyses, air-dried chromosome preparations were made from tissues macerated in one drop of 2% lacto-acetic orcein. For banding techniques and FISH, squashed preparations with 45% acetic acid were made, and then coverslips were removed after freezing the preparations by immersion in liquid nitrogen for a few seconds.

Chromosome banding

The distribution of heterochromatin was analyzed with Giemsa C-banding after treatments with 0.2M HCl for 15 min at 25 °C, 5% Ba(OH)₂ at 60 °C in a waterbath for 1 min and 2×SSC for 30 min at 60 °C (Sumner 1972). The GC- and AT-rich bands were detected with chromomycin A₃ (CMA₃) and 4'-6-diamidino-2-phenylindole (DAPI), respectively (Schweizer et al. 1983). The slides were stained with chromomycin A₃ (0.5 mg/ml in McIlvaine buffer, pH 7.0, containing 10 nM MgCl₂) for 60 min, washed with distilled water, stained with distamycin A (0.1 mg/ml) for 30 min, again washed and finally stained with DAPI (0.5 mg/ml) for 30 min. The slides were then washed with distilled water, mounted with a 1: 1 mixture of glycerol and McIlvaine buffer, pH 7.0, and kept in the dark for at least 3 days. Silver nitrate staining of active



Figure 1. a map of Brazil showing collection sites in northeastern and southern Brazil b *Tropidacris collaris* c *Tropidacris cristata grandis*.

Table 1. Collection sites of studied species.

Species	Number of specimens	Collection sites
<i>Tropidacris collaris</i>	4♀, 8♂ 4♀, 7♂	Refúgio Ecológico Charles Darwin, Igarassu, Pernambuco, Brazil 08°03.00'S, 35°13.00"W (DMS) Gurjaú, Cabo de Santo Agostinho, Pernambuco, Brazil 08°10'00"S, 35°05'00"W (DMS)
<i>Tropidacris cristata grandis</i>	5♀, 10♂	Iguaçu National Park, Foz do Iguaçu, Paraná, Brazil 25°37'40.67"S, 54°27'45.29"W (DMS)

nucleolus organizer regions (Ag-NOR) was performed according to Howell and Black (1980). Two drops of 1% aqueous gelatin solution with 0.25% formic acid and four drops of silver nitrate at 25% were placed onto the test slides, which were covered with coverslips and incubated for 7 min at 60 °C.

Fluorescence in situ hybridization (FISH)

In addition to the karyotype studies, genomic DNA from one male of each species was extracted from the muscle tissue sample. After proteinase K (20 mg/ml) digestion for three hours at 65 °C, phenol/Tris-HCl (pH 8.0) was added, followed by centrifugation and washing with phenol/Tris-HCl (pH 8.0) and chloroform-isoamyl alcohol. After an

additional centrifugation, chloroform-isoamyl alcohol was added. Then, DNA was precipitated with absolute ethanol for 12 hours at -20°C and eluted in TE 1/10 + RNase.

Unlabeled 18S rDNA and histone H3 gene probes were generated by polymerase chain reaction (PCR) using the following primers: 18S rDNAF 5'-CCTGA GAAACG-GCTACCACATC-3' and 18S rDNAR 5'-GAGTCTCGTTTCGTTATCGGA-3' (Whiting 2002); H3F 5'-ATATCCTTRGGCATRATRGTGAC-3' and H3R 5'-ATGGCTCGTACCAAGCAGACVGC-3' (Colgan et al. 1998). The probes isolated by PCR were labeled with digoxigenin-11-dUTP by PCR. Fluorescence *in situ* hybridization was performed according to Pinkel et al. (1986) with modifications. The slides were dehydrated in an alcohol series and washed in 15% formamide/0.2×SSC, pre-treated with DNase-free RNase (40 $\mu\text{g}/\text{ml}$ in 2×SSC) at 37°C for 1h and with pepsin (0.005% in 10 mM HCl) at 37°C for 30 min. Subsequently, they were fixed in 4% fresh paraformaldehyde, dehydrated in an alcohol series and air-dried. The chromosomes were then denatured in 70% formamide/2×SSC at 70°C for 5 min. The slides were treated with 30 μl of hybridization mixture containing 100 ng of labeled probe (4 μl), 50% formamide (15 μl), 50% polyethylene glycol (6 μl), 20×SSC (3 μl), 100 ng of calf thymus DNA (1 μl) and 10% SDS (1 μl). The material was denatured at 90°C for 10 min. Hybridization occurred overnight at 37°C in a humidified chamber. Post-hybridization washes were carried out in 2×SSC, 20% formamide in 0.1×SSC, 0.1×SSC and 4×SSC/0.2% Tween 20, all at 42°C . The probe was detected with a solution of 5% BSA and FITC-conjugated avidin (50:0.5, v:v). The post-detection washes were performed in 4×SSC/0.2% Tween 20 at room temperature. The slides were mounted with 25 μl of a medium composed of 23 μl of DABCO solution (1,4-diazabicyclo[2.2.2] octane (2.3%), 20 mM Tris HCl, pH 8.0, (2%) and glycerol (90%), in distilled water), 1 μl of 2 $\mu\text{g}/\text{ml}$ DAPI and 1 μl of 50 mM MgCl_2 .

All images were acquired with a Leica DM 4500 B microscope equipped with a DFC 300FX camera and Leica IM50 4.0 software, and optimized for best contrast and brightness with iGrafx Image software.

Results

Tropidacris collaris

The analysis of mitotic and meiotic chromosomes of *T. collaris* revealed $2n=24$, XX (Fig. 2a) and $2n=23$, X0 in females and males respectively. All chromosomes were acrocentric and arranged in three groups according to size: two large (L_1 - L_2), six medium-sized (M_3 - M_8) and three small pairs (S_9 - S_{11}). The X was the largest among the medium chromosomes. In male meiosis, the X univalent was positively heteropycnotic, as it was observed in diplotene and diakinesis (Fig. 3a, b).

Heterochromatic blocks revealed by C-banding were located in the pericentromeric regions of all chromosomes and M_4 showed the largest heterochromatic block. The medium-sized chromosomes carried small distal blocks, except for M_8 without

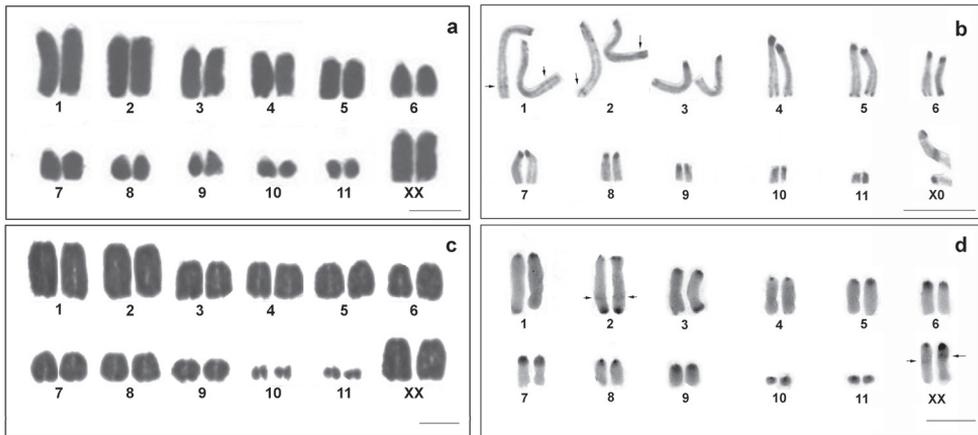


Figure 2. Karyotypes of the studied species. **a** female karyotype of *T. collaris*, conventional staining **b** male karyotype of *T. collaris*, C-banding **c** female karyotype of *T. cristata grandis*, conventional staining **d** male karyotype of *T. cristata grandis*, C-banding. Arrows indicate interstitial bands. Bar = 10 μ m.

distal blocks, and the X chromosome with a large distal block (Fig. 2b). Additionally, in meiosis, the bivalents L_1 , L_2 and M_4 exhibited small interstitial subterminal blocks (Fig. 3c).

The triple staining $CMA_3/DA/DAPI$ revealed CMA_3^+ blocks on the bivalents M_4 , S_{10} and S_{11} , the first block being of lower intensity (Fig. 3d). CMA_3^+ blocks, which were located in the bivalents S_{10} and S_{11} , coincided with the nucleolus organizer regions (NORs). However, a single individual was heterozygous for one of the NOR bivalents (Fig. 3d). DAPI showed homogeneous staining of all chromosomes (Fig. 3e). NORs were restricted to the distal regions of the bivalent S_{10} and pericentromeric region of S_{11} (Fig. 5a), both demonstrating nucleolar activity in all cells analyzed. In addition, Ag-NOR staining revealed a kinetochore marking in S_{10} (Fig. 5a) and another marking (presumably also indicating a nucleolus) at the opposite end of this bivalent.

Fluorescence *in situ* hybridization (FISH) revealed two markings in S_{10} and S_{11} coinciding with the NORs (Fig. 5b). However, the pattern visualized by FISH in the two analyzed samples showed differences in size of the signal involving one of the bivalent homologues, probably S_{10} that carried NORs, indicating a heterozygous condition of the rDNA site on this chromosome (Fig. 5b). FISH with histone H3 probe gene revealed sites on the bivalents M_6 , S_{10} and S_{11} , all located in the proximal position (Fig. 5c).

Tropidacris cristata grandis

All samples of *T. cristata grandis* exhibited $2n=23$ in males and $2n=24$ in females, featuring a sex chromosome system of the X0/XX type (Fig. 2c). Two pairs were of large size (L_1 - L_2); seven were medium-sized (M_3 - M_9) and two pairs were small-sized

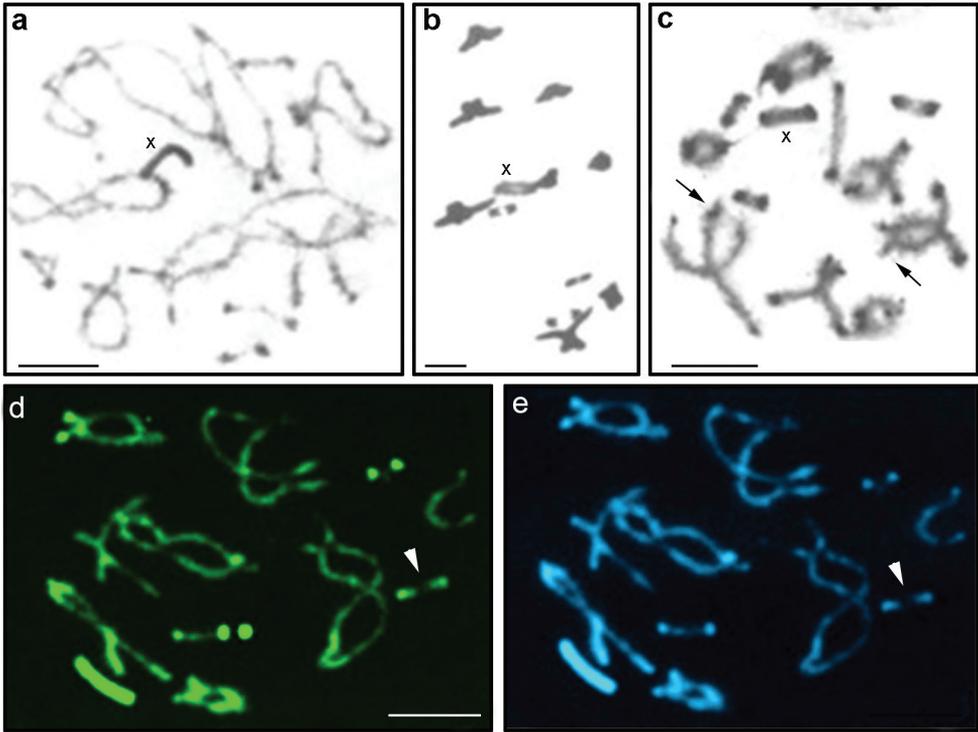


Figure 3. Meiotic stages of *T. collaris*. **a** pachytene **b** diakinesis **c** C-banding **d** CMA₃ staining **e** DAPI staining. Arrows indicate C⁺ subterminal blocks in larger pairs and in M₄. Arrowhead shows the heterozygous form. Bar = 10 μm.

(S₁₀-S₁₁); all chromosomes were acrocentric. The X chromosome was medium-sized and also acrocentric (Fig. 2c).

The analysis of meiotic cells in males revealed eleven bivalents corresponding to the autosomes at the pachytene stage; one positively heteropycnotic univalent (the X chromosome); and a structure in a particular bivalent pointing to a secondary constriction (Fig. 4a). At diplotene/diakinesis, eleven bivalents were present. One of them carried a secondary constriction, and the X chromosome remained univalent and positively heteropycnotic (Fig. 4b). Furthermore, one to three chiasmata per bivalent were observed, and one of the autosomes showed a degree of condensation very similar to the X chromosome demonstrating megameric characteristics. In anaphase I, it was possible to observe the correct migration of all autosomes and the presence of the X chromosome in only one of the two resulting cells. This corroborates the occurrence of an X0/XX system (Fig. 4c).

Heterochromatin revealed by C-banding was observed mainly as pericentromeric bands in all chromosomes. In pairs L₁, L₂, M₄, M₈, S₁₀ and S₁₁, these bands were small while they were more developed in the other pairs. Terminal heterochromatic bands were observed on the long arm of pairs L₁, L₂ and M₃. However, a heteromorphic

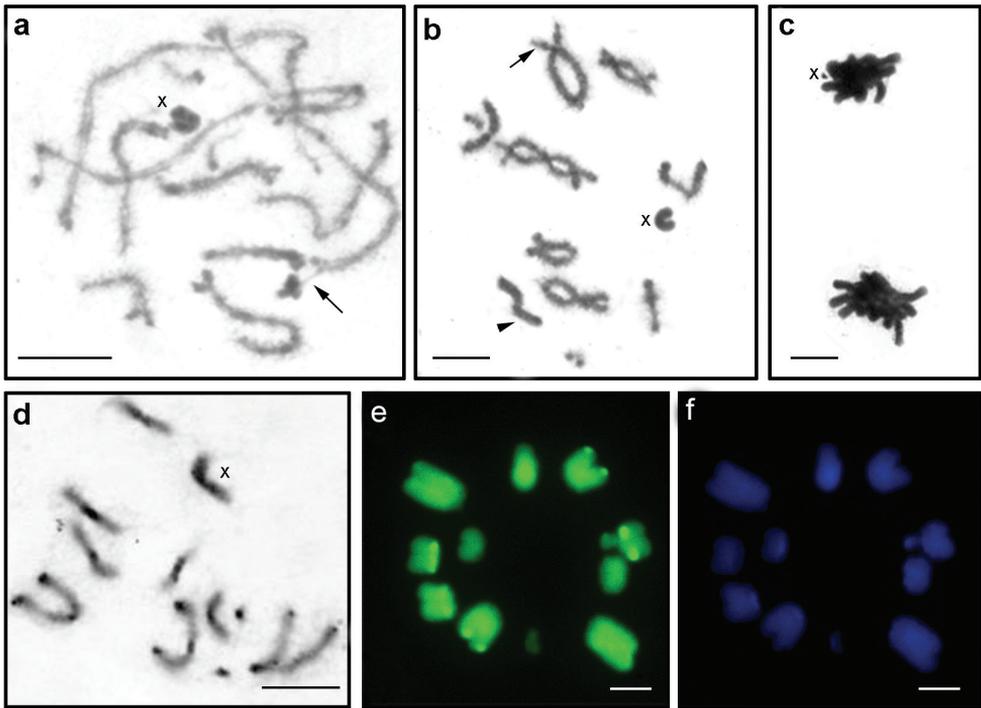


Figure 4. Meiotic stages of *T. cristata grandis*. **a** pachytene with positively heteropycnotic X chromosome **b** diplotene/diakinesis **c** two daughter cells at anaphase I with visible X chromosome in one of them **d** C-banding with pericentromeric and terminal blocks of heterochromatin in most chromosomes and positively heteropycnotic X chromosome **e** CMA₃ staining **f** DAPI staining. Arrows and arrowheads indicate secondary constrictions and the megameric chromosome respectively. Bar = 10 μm.

pair was found in two specimens, where one of the L₁ homologues did not carry this heterochromatic band. Furthermore, two pairs showed discrete bands on the long arm, one distal on pair L₂ and another (proximal) on the X chromosome (Fig. 2d). In meiocytes, heterochromatic blocks were detected in the pericentromeric and terminal regions, and a more dense heterochromatic region corresponded to the X chromosome (Fig. 4d).

At metaphase II, fluorochrome staining showed terminal GC-rich blocks on L₁ and L₂ pairs and on three other chromosomes with CMA₃⁺ blocks in the proximal regions (Fig. 4e). The AT-rich bands were more discernible than GC-rich bands and were observed only in the pericentromeric regions of five bivalents. In a medium-sized pair, this band was adjacent to a CMA₃⁺ region (Fig. 4f). A medium-sized chromosome carried a CMA₃⁺/DAPI band, in addition to the terminal CMA₃⁺ regions syntenic with the pericentromeric DAPI⁺ bands (Figs 4e and 4f).

Active NORs were found in one or two bivalents at pachytene (Fig. 5d). FISH with 18S rDNA probe detected rDNA clusters in two bivalents of medium size during metaphase II, M₅ and M₆, confirming occurrence of multiple NORs in the hetero-

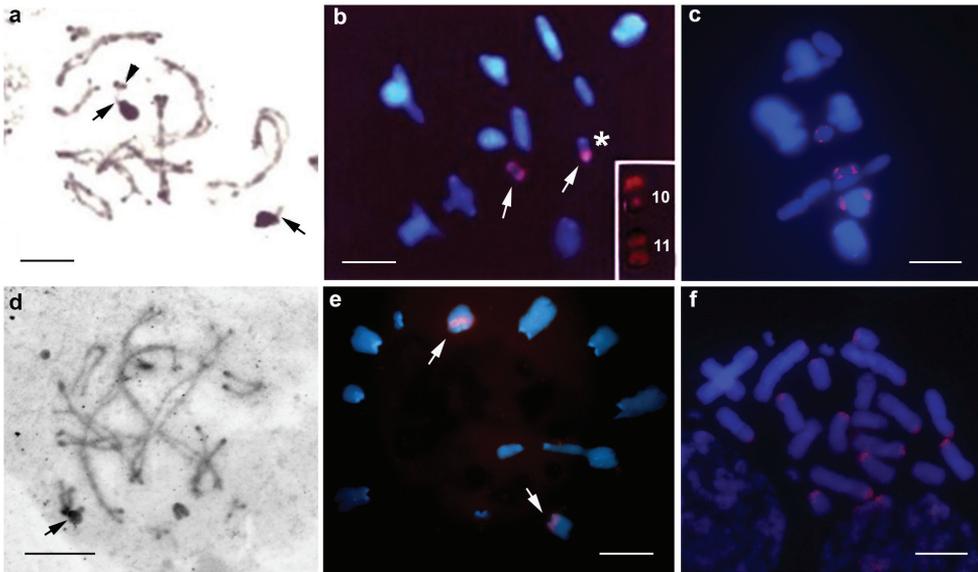


Figure 5. Mitotic and meiotic cells of *T. collaris* (a, b, c) and *T. cristata grandis* (d, e, f). a, d silver nitrate impregnation b, e FISH with 18S rDNA probe c, f FISH with histone H3 gene probe. Black and white arrows, arrowheads and asterisk indicate Ag-NOR bands, rDNA sites, pericentromeric regions and chromosome pair no. 10 respectively (heterozygous condition shown in the box). Bar = 10 μ m.

chromatic regions of this species (Fig. 5e). The histone H3 gene probes hybridized in the pericentromeric regions of most chromosomes, except for the two medium-sized and two small pairs (Fig. 5f).

Discussion

The observed diploid number ($2n\♂=23, X0$) and the overall structure of the karyotype containing only acrocentric chromosomes were identical in the two species. These karyotypes are similar to those reported for most species of Romaleidae (Mesa et al. 1982, Souza and Kido 1995, Loreto et al. 2005). However, in *Xestotrachelus robustus* (Bruner, 1911), pairs S_9 and S_{10} are meta- or submetacentric and originated by pericentric inversion. This species, therefore, retains the same chromosome number (Souza et al. 2003).

Although *T. collaris* and *T. cristata grandis* have the same chromosome number, they can differ in the karyotype structure. Both species have two pairs of large chromosomes. However, the karyotype of *T. collaris* contains six pairs of medium-sized chromosomes (M_3 - M_8) and three small pairs (S_9 - S_{11}). On the other hand, *T. cristata grandis* has seven medium-sized pairs (M_3 - M_9) and two small pairs (S_{10} - S_{11}). Furthermore, the conventional analysis of meiocytes revealed two other differences between these species. Specifically, *T. cristata grandis* has a large chromosome with a secondary constriction, and a megameric chromosome. Grasshoppers of the family Romaleidae have conserved

karyotypes, i.e., they reveal extensive uniformity in the chromosome number and chromosomal morphology. However, we found an extensive interspecific variability as regards chromosome banding in the species studied, which indicates a wide variation in the distribution of repetitive DNA sequences. Such variation was also observed in several other members of the group (Vilardi 1988, Souza and Silva-Filha 1993, Souza and Kido 1995, Souza et al. 1998, Pereira and Souza 2000, Souza et al. 2003).

T. collaris and *T. cristata grandis* have pericentromeric C-bands in all chromosomes. However, we also noted some differences in the distribution of other heterochromatic blocks (Figs 2b and d). Specifically, a pair of chromosomes of *T. collaris* carried a large pericentromeric block of heterochromatin. On the other hand, similar blocks were detected in five pairs of *T. cristata grandis* chromosomes, demonstrating that increase in the amount of heterochromatin appears to be more active in the latter species. Moreover, differences in the distribution of other bands were also observed. In *T. cristata grandis*, the structure of L₁ chromosome varied among different individuals. For example, one of the homologues did not carry the terminal heterochromatic band. This could be explained in two ways: (i) either by a deletion of this terminal heterochromatic region; or (ii) this chromosome could represent the initial phase of heterochromatinization, where only one of the homologues has the terminal heterochromatic segment. Judging from the heterochromatin distribution in the two other species, these divergences can originate by amplification, multiple replications, unequal crossing-over, accumulation and elimination (John 1988).

Allopatric speciation usually occurs after the relatively long geographical isolation between populations. Consequently, these populations accumulate genetic differences that can cause reproductive incompatibilities (Presgraves 2010). These genetic differences may be related either to gene mutations or to changes in the chromosome constitution, such as heterochromatin spreading in both autosomes and sex chromosomes (Presgraves 2010, Molina et al. 2012, Pucci et al. 2014). Schweizer and Loidl (1987) proposed that heterochromatin transfer between equilocal regions might occur between non-homologous chromosomes of similar size due to the positioning of these chromosomes in the nucleus bouquet configuration. The transfer of the pericentromeric heterochromatin probably happened in *Tropidacris* species studied in this paper. The expansion of heterochromatin could also occur due to transposable elements, which are genetic determinants of heterochromatin formation in different organisms (Grewal and Jia 2007). Then, geographical isolation occurred in the ancestral populations of this genus, as proposed by Carbonell (1986). This, in turn, enabled heterochromatinization that led to differences in the chromosome constitution observed in the two species. The action of geological forces added to the current fragmentation of the environment and, therefore, allowed the formation of allopatric populations, promoting speciation in the genus.

Other differences were found between the two species with respect to the base content of DNA that constitutes heterochromatin. CMA₃⁺ blocks (GC-rich) were observed in three chromosomes of *T. collaris*, while *T. cristata grandis* showed a higher number of bands. These results indicate that, in addition to the expansion of the heterochromatin, its composition has also been modified in terms of base pairs. The occurrence

of DAPI⁺ heterochromatin (AT-rich) in *T. cristata grandis* also differentiates it from *T. collaris*, since the karyotype of the latter species does not have AT-rich regions. The same is true for the various members of this family studied to date (Loreto et al. 2008, Rocha et al. 2011, Bueno et al. 2013). Likewise, the DAPI⁺ blocks of the chromosomes M₃ and M₅ of *T. cristata grandis* were syntenic with CMA₃⁺ blocks. These results indicate the presence of at least three different compositions of heterochromatin in this species; GC-rich, AT-rich, and that with interspersed AT and GC-rich segments. In *T. collaris*, there are two types of heterochromatin composition: a GC-rich one and another neutral for AT, e.g., all chromosomes with homogeneous staining. These results reinforce the differences between the two species, where the pattern found in *T. collaris* is more similar to that observed in most species of Romaleidae (Souza et al. 1998, Pereira and Souza 2000, Souza et al. 2003, Anjos et al. 2013). *T. collaris* has a wider distribution, and in *T. cristata grandis* it is geographically more restricted. Thus, gene flow is higher in *T. collaris* and, therefore, chromosomal rearrangements are not easily established within different populations of this species.

Most studies of histone H3 genes in grasshoppers reveal a localization of these sequences on a single pair of chromosomes (Cabrero et al. 2009, Cabral-de-Mello et al. 2011a, 2011b, Regueira-Neto et al. 2013). In *T. collaris* and *T. cristata grandis*, these genes were observed in more than one pair, and histone H3 genes were always found in terminal regions of 14 autosomes and the X chromosome in the latter species. A similar situation was reported for the grasshoppers *Abracris flavolineata* (De Geer, 1773) (Bueno et al. 2013) and *Rhammatocerus brasiliensis* (Bruner, 1904) (Oliveira et al. 2011). The difference in the distribution of histone genes leads to the assertion of a possible amplification of this gene family. Moreover, the distribution of the histone genes corresponds to heterochromatin location and may show an intercalary distribution of these sequences. This heterochromatic region consists of highly condensed chromatin, where few mRNAs are produced and there is abundance of repetitive elements, such as satellite DNA and transposons (Pikaard and Pontes 2007). The association between genes and repetitive sequences, such as histone H3 genes, could result in variation in the number of copies of these genes. Thus, an increase in unequal exchanges and gene duplication might occur (Zoldos et al. 1999, Hamon et al. 2009). Moreover, the transposable elements of the heterochromatic regions could contribute to the dispersion of histone genes to several chromosomes, as observed in different genetic sequences (Sczepanski et al. 2013, Barbosa et al. 2015).

The wide range of variation in heterochromatin among the samples of the two species, in addition to the distribution of GC-rich blocks and co-localization with H3 histone genes in *T. cristata grandis*, is an exceptional feature in grasshoppers. Heterochromatin has the ability to spread to different regions and influence gene expression, leading to silencing of some genes and preventing recombination between them (Gottlieb and Esposito 1989, Grewal and Jia 2007). The presence of multiple copies of histone H3 genes in the heterochromatic regions may indicate that these copies are silenced. The co-localization with CMA₃⁺ regions is indicative of silencing, since DNA methylation occurs preferentially in GC-rich regions and also influences gene silenc-

ing (Newell-Price et al. 2000, Schotta et al. 2002), thus providing a balance in the processes of expression of the histone H3 genes.

The NORs were observed in two chromosome pairs in both species, although these pairs are different. While *T. collaris* has NORs on two small pairs, these structures were observed on medium-sized chromosomes of *T. cristata grandis*. Regueira-Neto et al. (2013) suggested that the location of NORs on medium-sized autosome pairs is the ancestral location of the 45S rDNA genes in Romaleidae. Thus, location of rDNA sites on small chromosome pairs of *T. collaris*, as well as the presence of multiple copies on more than one autosomal pair indicate the occurred rearrangements and amplification of the sequences associated with the spread of heterochromatic regions.

The data presented in this study demonstrate karyotype conservation regarding the chromosome number and morphology in both species of *Tropidacris* when compared to other species of Romaleidae. However, they indicate that *T. cristata grandis* has an extremely diverse karyotype in terms of the presence and distribution of heterochromatic blocks and differentiation in the localization of histone H3 genes, showing karyotypic differences from *T. collaris*. While *T. collaris* is widely distributed in Brazil, *T. cristata grandis* has a restricted geographical distribution within isolated fragments of the rainforest. Originally, the Atlantic Forest extended along almost the whole east coast of Brazil, with extensive incursions into the inner parts of the country (Morellato and Haddad 2000). Nowadays, it is reduced to a set of small remaining fragments, usually less than 100 hectares, which are isolated and subject to intense edge effect (Ribeiro et al. 2009). The restricted distribution of this species is associated with the devastation of these environments and can lead to geographical isolation of the resulting populations. This isolation can promote establishment of the above-mentioned karyotypic changes and launch the speciation process in *T. cristata grandis*, which becomes increasingly divergent from the other species of the genus.

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Contributions to cytogenetics of *Plectranthus barbatus* Andr. (Lamiaceae): a medicinal plant

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Abstract

Accessions of *Plectranthus barbatus* (Lamiaceae), a medicinal plant, were investigated using a cytogenetic approach and flow cytometry (FCM). Here, we describe for the first time details of the karyotype including chromosome morphology, physical mapping of GC rich bands (CMA₃ banding), as well as the mapping of 45S and 5S rDNA sites. All accessions studied showed karyotypes with $2n = 30$ small metacentric and submetacentric chromosomes. The CMA₃ banding and fluorescent *in situ* hybridization techniques revealed coincidence between CMA₃ bands and 45S rDNA sites (6 terminal marks) while for the 5S rDNA were observed 4 subterminal marks no coincident with CMA₃ marks. For nuclear genome size measurement, the FCM procedure provided histograms with G₀/G₁ peaks exhibiting CV between 2.0–4.9 and the mean values obtained for the species was $2C = 2.78$ pg, with AT% = 61.08 and GC% = 38.92. The cytogenetic data obtained here present new and important information which enables the characterization of *P. barbatus*.

Keywords

AT/GC content, chromosome number, heterochromatin, genome size, molecular cytogenetics

Introduction

The genus *Plectranthus* L' Herit. (Lamiaceae) contains nearly 300 species distributed in tropical Africa, Asia, Australia and Brazil (Lukhoba et al. 2006, Alasbahi and Melzigh 2010). Many species show important medicinal properties and a large variation in morphology, chemistry as well as in the chromosome number, ranging from $2n = 14$ to $2n = 84$ (Morton 1962, De Wet 1958, Lukhoba et al. 2006, Alasbahi and Melzigh 2010).

One of the most important species traditionally used in folk medicine, *Plectranthus barbatus* Andr., shows a large morphological variation and is also commonly cited by innumerable synonyms such as *P. forskohlii* Briq., *P. forskalaei* Willd., *P. kilimandschari* (Gürke) H. L. Maass., *P. grandis* (Cramer) R. H. Willemse, *Coleus forskohlii* Briq., *C. kilimandschari* Gürke ex Engl., *C. coeruleus* Gürke, *C. comosus* A. Rich., and *C. barbatus* (Andr.) Benth (Lukhoba et al. 2006, Alasbahi and Melzigh 2010).

Popularly, *P. barbatus* is mainly used for liver disturbance, respiratory disorders, heart diseases and certain central nervous system disorders, being also used as hypotensive and antispasmodic (Alasbahi and Melzigh 2010). Due to these broad applications, numerous chemical and pharmacological studies have been done showing forskolin also called coleonol as the major active component (Lukhoba et al. 2006, Alasbahi and Melzigh 2010).

In spite of intensive pharmacological studies, few studies have been done about biological aspects of the species. Considering the medicinal importance of *P. barbatus* and a large number of synonyms reported, basic information such as karyotypic traits are very important, helping the correct plant identification and also the production of commercial varieties in breeding programs (Sousa et al. 2009, Ferreira et al. 2010, Sousa et al. 2010, Pierre et al. 2011, Sousa et al. 2012, Sousa et al. 2013, Reis et al. 2014, Viccini et al. 2014). Regarding cytogenetic studies, only the chromosome number and meiosis behavior were reported so far (De Wet 1958, Morton 1962). Detailed data such as chromosome banding and molecular cytogenetic traits are not available for *Plectranthus* species.

The aim of the present work was to describe new chromosome markers for *P. barbatus*, by using chromosome banding and molecular cytogenetic techniques. The genome size and AT/GC content by flow cytometry were also reported to help the characterization of different cytotypes of *P. barbatus* as well as to understand the taxonomy and evolution of the genus *Plectranthus*.

Material and methods

Plant material

Five accessions of *Plectranthus barbatus* were collected at Juiz de Fora, Latitude: 21°45'51"S and Longitude: 43°21'01"W, Minas Gerais, Southeast Brazil and cultivated in a greenhouse of Federal University of Juiz de Fora. The herbarium voucher

specimens of each accession were deposited at the CESJ Herbarium of Federal University of Juiz de Fora with following numbers: PB 2324, PB 2325, PB 2326, PB 2327 and PB 2328.

Preparation of mitotic spreads

Roots tips were pre-treated with 8-hydroxyquinoline solution (0.003 M) at room temperature for 7h and then fixed in ethanol and acetic acid (3:1 v/v) for 24h at -20 °C. Root meristems were submitted to enzymatic maceration (4% Celullase: 40% Pectinase) for 5h at 37 °C. The slides was prepared according to Carvalho and Saraiva (1993, 1997).

Determination of morphological chromosome parameters

Chromosome length, short and long arms and ratio between chromosome arms (AR) were measured on 5 well-spread metaphases for each accession using the CellSens software (Olympus, Tokyo, Japan). Chromosome classification was done according to Levan et al. (1964). The ideogram were drawn based on centromeric index and arranged in the decreasing size order.

Molecular cytogenetics

Fluorescence *in situ* hybridization (FISH) was performed using the probe pTA71 from *Triticum aestivum*, which contain a 9kb EcoRI fragment including the 18S – 5.8S – 25S rRNA gene and intergenic spacer regions (rDNA) (Gerlach and Bedbrook 1979) and 5S probes from *Zea mays* (D.-H. Koo and J. Jiang, University of Wisconsin, unpublished data), kindly provided by Dr. J. Jiang. Each probe was labeled with digoxigenin by nick translation and then hybridized according to Jiang et al. (1995) with minor modifications. The hybridization mixture was denatured at 85 °C for 10 min and immediately transferred to an icebox. The slides were denatured at 85 °C for 1 min and treated with a series of alcohol washes (70%, 90%, and 100% ethanol for 5 min each). The hybridization mixture was then added to the slides and the chromosomes allowed to hybridize at 37 °C for 48 h in a humidified chamber. Posthybridization washes were carried out using 2 × SSC buffer (0.3 mol/L sodium citrate, 0.03 mol/L sodium chloride, pH 7) and 1 × PBS buffer (0.136 mol/L sodium chloride, 0.27 mol/L potassium chloride, 0.1 mol/L dibasic sodium phosphate, 0.2 mol/L monobasic potassium phosphate, pH 7.4). Probes were detected with anti-DIG conjugate with rhodamine (Sigma) and postdetection washes were performed using 1 × TNT buffer (0.1 mol/L Tris, 0.15 mol/L sodium chloride, 0.05% Tween-20) and 1 × PBS at room temperature. Chromosomes were counterstained with 2 µg/mL of DAPI (Sigma). The

slides were mounted in Vectashield (Vector, Burlingame, California, USA), and some samples were rehybridized after discoloration in 100% ethanol for 24h. Good metaphases were captured in an Olympus DP72 digital camera and images with DAPI, 45S and 5S signals were merged using CellSens software (Olympus, Tokyo, Japan). Chromosomes were observed using an epifluorescence microscope (Olympus BX 51) with appropriate filter set (Olympus, Tokyo, Japan).

Chromosome banding

The chromosome banding was performed according Schweizer (1976). Aged slides were stained with chromomycin A₃ (0.5 mg/mL) for 1 h, dystamycin (0.1 mg/mL) for 30 min and 2-4 diamidino-2-phenylindole (2 µg/mL) for 30 min. The slides were mounted in McIlvaine's pH 7.0 buffer-glycerol (1:1 v/v). For this analysis five metaphases of each accession were observed and captured in an Olympus DP72 digital camera. The chromosomes were observed using an epifluorescence microscope (Olympus BX 51) with appropriate filter set.

Flow Cytometry (FCM)

Nuclear DNA content was determined according to the method of Galbraith et al. (1983). Approximately 20–30 mg of young and fresh leaves for each accessions of *P. barbatus* and the same amount of young foliar tissue of standard references *Zea mays* CE-777 were chopped on ice with 1 mL of OTTO I lysis buffer solution (Otto 1990) supplemented with 50 µg mL⁻¹ RNase. The suspension was filtered through 40nm mesh into 2 mL microcentrifuge tube and centrifuged at 1,100 rpm for 5 minutes. The pellet was incubated in 100 µL OTTO I lysis buffer for 10 minutes and then was added 1.4 mL of OTTO I: OTTO II (1:2 v/v) buffers. The sample were homogenized and stained with 50 µg mL⁻¹ of propidium iodide (PI) to determine the total DNA content. AT/GC composition was determined by adding DAPI (4,6-diamidino-2-phenylindole) 4 µg mL⁻¹ to the samples. At least 10,000 nuclei were analyzed per sample in a FACSCantoII (Becton Dickinson) flow cytometer. The histograms were analyzed using Flowing 2.5.1 software (<http://www.flowingsoftware.com>).

The DNA nuclear amount (pg) of each sample was estimated by the relative fluorescence intensity of the sample and the internal reference standard (*Zea mays* 5.43). Each accession was measured three times following the equation (Dolezel 2003):

$$DNA\ content\ of\ sample = \frac{PIFI\ of\ sample \times DNA\ content\ of\ standard}{PIFI\ of\ standard}$$

where *PIFI* is the fluorescence intensity of cells stained with propidium iodide in G1 stage.

The AT percentage of *P. barbatus* was measured in relation to *Zea mays* reference standard, following the equation described by Godelle et al. (1993):

$$AT_{sample} = AT_{standard} \left(\frac{R_{DAPI}}{R_{PI}} \right)^{1/r}$$

where *R* is the ratio of fluorescence intensity between the peak of *P. barbatus* and *Zea mays*, and *r* (binding length) = 3 for DAPI dye (Meister and Barow 2007). The percentage of the complementary bases was calculated as GC% = 100 – AT%.

Results

The accessions showed symmetrical karyotype, all with 2n = 30. Fourteen chromosomes showed centromeres at the median (m, AR = 1–1.7) and one of them at submedian region (sm, AR = 1.71–3.0) (Table 1). No secondary constrictions were observed. Chromosome lengths ranged from 2.51–1.86 µm (Table 1) and the Karyotype formulae (KF) was KF = 14m+1sm.

Relative chromosome length revealed that the larger chromosome represented around 7.91% of the genome size and the shortest one 5.86% (Table 1).

The 45S rDNA signal were observed in three chromosome pairs on the terminal portion (two in the short arms of chromosomes 6 and 10, and one in the long arm of chromosome 11) (Fig. 1 B1–B3), while 5S rDNA signals were observed in subterminal portion of two chromosome pairs, in the short arm of chromosomes 9 and 12, respectively (Fig. 1 A1–A3). The 45S rDNA sites showed greater bands when compared with those ones observed for 5S rDNA sites, which showed pairs of little dots (Fig. 1 A2–A3 and B2–B3).

No centromeric, interstitial or terminal DAPI bands were observed. However, fluorochrome staining with CMA₃ revealed bands on three chromosome pairs, which were also DAPI negative. Heterochromatin blocks correspond to 0.37% of the total haploid complement. The observed bands showed similar size and bright, all of them at terminal regions and coincident with 45S rDNA marks, on the short arm of chromosome 6 and 10, respectively, and on the long arm of chromosome 11 (Fig 1C, D). No additional CMA bands were observed in any of the chromosome pairs.

Regarding to the nuclear genome size estimation, the flow cytometry (FCM) technique provided high quality histograms with G₀/G₁ peaks showing CV = 2,0–4,9 . The 2C DNA content estimated for the species was 2C = 2.78 pg (Table 1). Taking into account that 1pg = 978 Mpb (Dolezel et al. 2003), and combining cytometric and cytogenetic data the largest chromosome (chromosome 1) corresponds to 107.452 Mpb (~7% of the genome), while the smallest one (chromosome 15) corresponds to 79.605 Mpb (~6% of the genome). By using FCM *P. barbatus* genome (2C) possess 2714.148 Mpb (Table 1).

In addition, PI and DAPI fluorochromes index allowed, for the first time, the estimation of base composition of the genome of *P. barbatus*. The percentage of base was 61.08% for AT and 38.92% for GC. The representative histograms of DNA content and base composition can be seen in Figure 1E.

Table 1. Chromosome morphometry of *P. barbatus* and estimative of DNA content for each chromosome.

Chromosome	Relative length (%)	Absolute length (µm)	Short arm length (µm)	Long arm length (µm)	Arm ratio	Classification	DNA pg/chromosome	Mpb/chromosome
1	7.91	2.512	1.127	1.385	1.22	m	0.109	107.453
2	7.45	2.362	0.995	1.367	1.37	m	0.103	101.204
3	7.07	2.247	0.900	1.347	1.49	m	0.098	96.042
4	7.05	2.242	1.050	1.192	1.13	m	0.097	95.770
5	6.97	2.220	0.885	1.335	1.50	m	0.096	94.683
6	6.68	2.120	0.980	1.140	1.16	m	0.092	90.744
7	6.65	2.112	0.995	1.117	1.12	m	0.092	90.336
8	6.55	2.080	0.940	1.140	1.21	m	0.090	88.978
9	6.46	2.047	0.815	1.232	1.51	m	0.089	87.755
10	6.43	2.047	0.842	1.205	1.43	m	0.089	87.348
11	6.41	2.040	0.837	1.202	1.43	m	0.089	87.076
12	6.25	1.980	0.825	1.155	1.40	m	0.086	84.903
13	6.18	1.960	0.702	1.257	1.79	sm	0.085	83.952
14	5.98	1.895	0.812	1.082	1.33	m	0.083	81.235
15	5.86	1.865	0.857	1.007	1.17	m	0.081	79.605

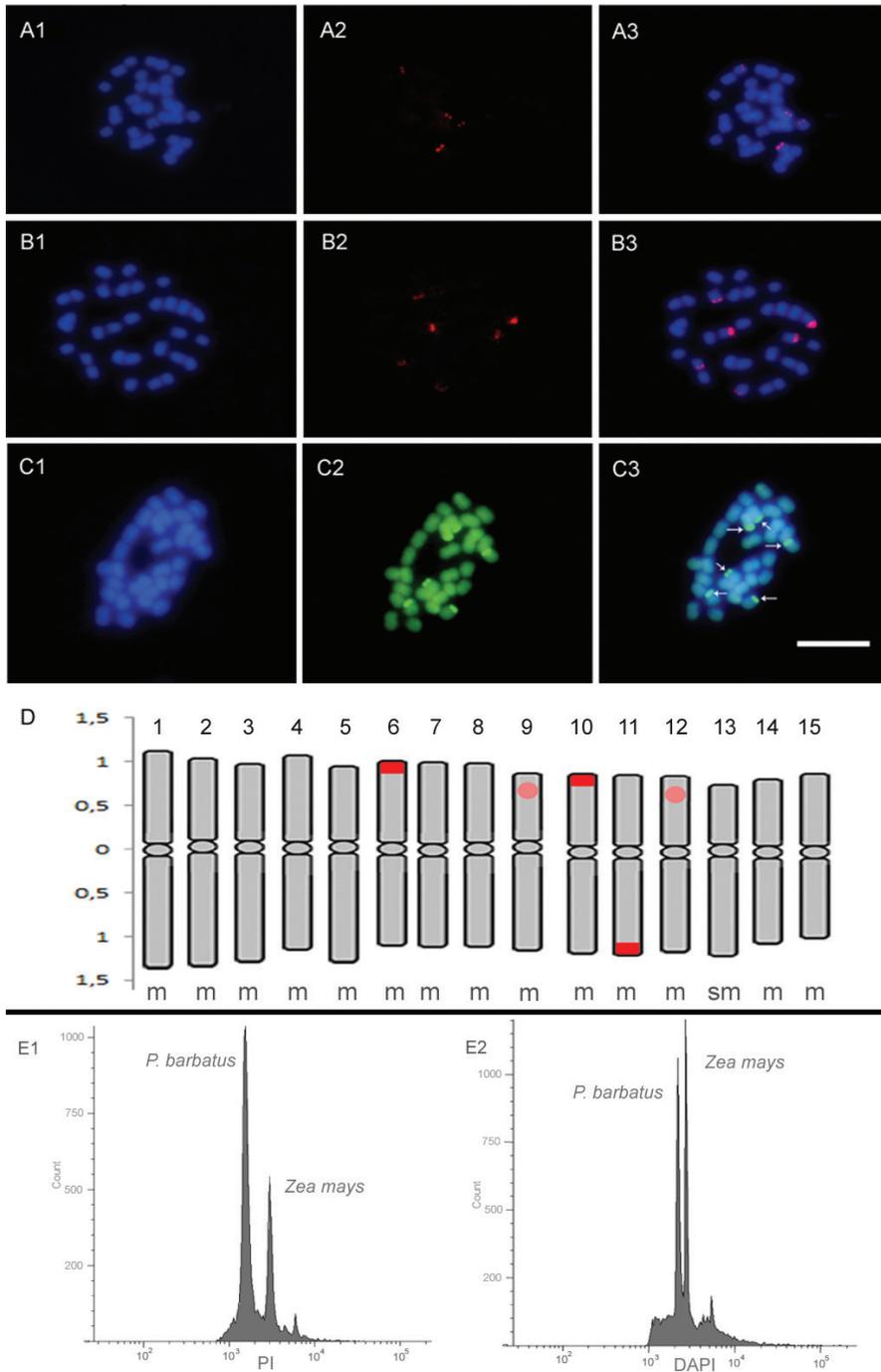


Figure 1. Representative metaphases of 5S rDNA (**A1, A2, A3**) and 45S rDNA (**B1, B2, B3**), DAPI/CMA₃ banding (**C1, C2, C3**), Ideogram of *P. barbatus* (**D**) (light red circle = 5S rDNA, dark red rectangle = 45S rDNA), Flow cytometry histograms (**E1** = propidium iodide - DNA total amount, **E2** = DAPI - AT content). Bar = 5 μ m.

Discussion

The genus *Plechtrantus* has a great variability of chromosome numbers. Although the basic chromosome number for most of the species is $x = 7$, and $2n$ chromosome number is 28, some species show secondary basic chromosome numbers (6 and 8) (De Wet 1958, Morton 1962). Additionally, intraspecific chromosome numbers variations are common in the genus. Morton (1993) described, for example, the following aneuploid numbers: $2n = 26$ and 28 for *P. assurgens* (Baker) J. K. Morton and *P. glandulosus* Hook, $2n = 28$ and 30 for *P. tenuicaulis* (Hook. f.) J. K. Morton, while for *P. amboinicus* (Lour.) Spreng, Thoppil (1993) described a large variation of somatic numbers ($2n = 16, 24, 30, 32, 34$ and 48), indicating that polyploidy events, in association with aneuploidy might contributed to the genus complexity.

Many authors suggested that *P. barbatus* can be cytologically considered as a species with different cytotypes that include a possible aneuploid series with $2n = 28, 30, 32$ and 34 described so far (Reddy 1952, Riley and Hoff 1961, Saggoo and Bir 1983). In our study all individuals showed $2n = 30$, corroborating one of the numbers previously described (Cherian and Kuriachan 1981, Saggoo and Bir 1983, Bahl and Tyagi 1988, Thoppil 1993). If we consider $x = 7$ as the main basic chromosome number for the genus *Plechtrantus*, as was suggested by most of the authors, and the same number as the basic chromosome number for *P. barbatus*, $2n=30$ could be a consequence of both polyploidy and aneuploidy events however meiotic behaviour studies are necessary to understand this condition.

Chromosome length and chromosome classification of *P. barbatus* here observed is very similar to those ones already described for other cytotypes of *P. barbatus* (De Wet 1958, Morton 1962, Cherian and Kuriachan 1981, Saggoo and Bir 1983, Bahl and Tyagi 1988, Thoppil 1993). Nevertheless, some karyotypic formulae described are different from our data indicating that the genome of the species, apart from chromosome number variation, is also very instable regarding the chromosome structure (De Wet 1958, Morton 1962, Bahl and Tyagi 1988, Thoppil 1993). Chromosome morphological alterations such as deletions, duplications, inversions, transpositions and translocations may have occurred independently over time in different accessions of *P. barbatus*. These rearrangements are very common in polyploids due to the genetic redundancy observed after the genome duplication, event known as “genome shock” (Lim et al. 2008, Reis et al. 2014). The individuals with extra copies of sequences/genes in an attempt of genome restructuring and adaptation undergo several modifications resulting in karyotype and phenotypic changes (Parisod et al. 2009, Soltis and Soltis 2009, Lipman et al. 2013). In *Lathyrus nervosus* Lam. (Fabaceae), for example, it was observed karyotypic variations in different populations although the individuals showed the same chromosome number (Chalup et al. 2012).

The cytomolecular data here observed is the first relate for the genus. The number of 5S rDNA probes observed was in according to the expected number. Nevertheless, two additional marks of 45S rDNA was detected (6 instead of 4) reinforcing the hypothesis that chromosome structural rearrangements such as duplication, trans-

locations and transpositions events might occurred after the chromosome doubling, increasing the number of 45S rDNA sites. Similar result was reported for *Byblis rorida* Lowrie & Conran ($2n = 16$) cytotypes (Fukushima et al. 2011). The observation of some individuals with additional sites of rDNA, suggested an increase in the number of the locus through structural rearrangements, since it was not observed any difference in chromosome number among the individuals investigated (Fukushima et al. 2011).

Alternatively, additional chromosomes (from unbalanced gamete) may also explain the number of 45S rDNA sites here observed for *P. barbatus*. Several authors have been discussed the stability of 45S and 5S rDNA (numbers, size and position) in the cytotypes formation. Reis et al. (2014), for example, described a polyploid complex for *Lippia alba* (Verbenaceae) and suggested that 45S rDNA sites are very variable (in general, the expected number of sites was not observed in polyploids due to deletions). On the other hand, taking the monoploid number of sites as reference, the 5S rDNA was more stable following the expected number according to the ploidy level. In the common bean *Phaseolus vulgaris* L. (Fabaceae), Pedrosa-Harand et al. (2006) observed that the number of 45S rDNA sites varied from 6 to 18 per accession. According to the authors, amplifications and deletions would be the probable reason of the wide variation observed. Regarding to the size of rDNA sites, some differences may be due to the partial amplification and deletion of some sites, already described as a common phenomenon in ribosomal sites of plant chromosomes (Roa and Guerra 2012).

Flow cytometry analysis indicated that the *P. barbatus* genome size is relatively small comparing with other Lamiaceae species. Taking all estimations described so far from 25 genera, the 1C value ranged from 0.28 to 6.24 pg. (Bennett 1972, Galbraith et al. 1983, Olszewska and Osiecka 1983, Ohri and Kumar 1986, Suda et al. 2003, Ohri et al. 2004, Rosenbaumová et al. 2004, Suda et al. 2005, Schmidt-Lebuhn et al. 2008, Kubesoal et al. 2010, Mahdavi and Karimzadeh 2010, Siljak-Yakovlev et al. 2010, Temsch et al. 2010, Bainard et al. 2011, Vesely et al. 2011). The present study reported is the first DNA content estimation for *P. barbatus* and also the first estimation for the genus *Plectranthus*.

Although there are several estimations of plant genome sizes, few of them reported the AT/GC genome composition, being the most detailed studies performed by Meister and Martin (2007) and Smarda and Bures (2012). Recently the average of GC composition for different kingdoms was reported begin possible to observe that monocots showed 45.15% of GC while dicots showed 34.36% of the same bases (Li and Du 2014). In *P. barbatus*, GC content estimation was quite close to the most of dicots (Carels 2005). It was also possible to observe that part of the GC base composition of *P. barbatus* genome (1.18%) corresponds to the bright blocks of CMA₃ constitutive heterochromatin. This is also the first report of chromosome banding data for the genus.

In addition to understand the biology and the evolution of plant species, the characterization of chromosome number and DNA content can be very interesting, especially when different cytotypes had been described for a medicinal species. Studying different accessions of *Lippia alba* (Mill.) N. E. Brown (Verbenaceae), an important

medicinal plant in Brazil, it was observed that different chemotypes, but morphologically similar, showed different major component of the essential oil (Viccini et al. 2014). While diploids and tetraploids possess citral as the major component, triploids cytotypes had linalool. Considering that linalool and citral have different medicinal applications, the characterization of these plants is very important, helping the correct medicinal use of the species (Viccini et al. 2014, Reis et al. 2014).

In addition to the new data here described more species of the genus *Plecthrantus* and of the Lamiaceae family should be investigated once no detailed cytogenetic data is available. The increase in the number of taxa will be very important for a better understanding of the biology and the evolutionary relationship within this important medicinal plant group. Other cytotypes and possible chemotypes of *P. barbatus* are up to know under-characterized.

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