

Cytogenetic studies of European Pulvinariini (Homoptera: Coccidae)

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Abstract. Cytogenetic characters and reproductive biology of 12 species from 9 genera of the tribe Pulvinariini are discussed. *Acanthopulvinaria orientalis* (Nasonov, 1908) (2n = 18), *Chloropulvinaria aurantii* (Cockerell, 1896) (2n=26), *Ch. floccifera* (Westwood, 1870), *Eupulvinaria peregrina* Borchsenius, 1953, *Protopulvinaria pyriformis* (Cockerell, 1894), *Phyllostroma myrtilli* (Kaltenbach, 1874) (all with 2n=16), and *Rhizopulvinaria variabilis* Borchsenius, 1952 (2n=28) are studied for the first time. *Pulvinaria regalis* Canard, 1968, **syn. n.** is placed in synonymy under *Pulvinaria vitis* Linnaeus, 1758.

Key words: scale insects, soft scales, karyotypes, sex-ratios, parthenogenesis.

INTRODUCTION

The tribe Pulvinariini Targioni Tozzetti, 1868 has a world-wide distribution and comprises 19 genera and about 215 species (Hodgson, 1994; ScaleNet, www.sel.barc.usda.gov/scalenet/scalenet.htm, on October 10, 2008). However the number of species and genera are disputable. There are two principal systems of the tribe developed by Borchsenius (1957) and Hodgson (1994); they both are based on adult female morphology. In this paper we follow the taxonomic conception by Borchsenius (1957), because it appears more convenient for the practical work on the group. Unfortunately, there are no paleontological or any other taxonomically significant data for clarifying the Pulvinariini phylogeny. In our opinion, specific reproductive and cytogenetic characters of pulvinariins can throw light on some disputable questions of the group phylogeny, but our knowledge on the cytogenetics and reproductive biology of the group is pres-

ently very limited and restricted mainly to the European fauna.

One of the main characters differing the Pulvinariini from other tribes of Coccidae is the presence of a woolly ovisac behind the body of an oviparous female with usually nude dorsum of female body and the absence of dorsal tubular ducts. The last character differs Pulvinariini from the subfamily Eropeltinae, some species of which are very morphologically similar with pulvinariins. However, some species traditionally placed in the Pulvinariini, for example, *Chloropulvinaria aurantii* (Cockerell, 1896) and *Phyllostroma myrtilli* (Kaltenbach, 1874), have intermediate state of the character discussed, demonstrating small number of dorsal tubular ducts and not dense wax secretions all over the dorsum or only on its posterior part. We prefer assigning these species to the Pulvinariini till a better justified system of Coccidae is elaborated.

Until our studies, only 5 species of Pul-

Table 1. Chromosomal numbers of Pulvinariini.

Species name	2n	Reference [locality]
<i>Acanthopulvinaria orientalis</i> (Nasonov)	18	Gavrilov, 2007a, b [Astrachan, Russia]
<i>Chloropulvinaria aurantii</i> (Cockerell)	26	Gavrilov, 2007 and present paper [Sochi, Russia]
<i>Ch. floccifera</i> (Westwood)	16	Gavrilov, 2007 and present paper [Crimea, Ukraine]
<i>Ch. polygonata</i> (Cockerell)	18	Moharana, 1990 [India]
<i>Ch. psidii</i> Maskell	14	Moharana, 1990 [India]
<i>Eupulvinaria peregrina</i> (Borchsenius)	16	Gavrilov, 2007 and present paper [Sochi, Russia]
<i>Phyllostroma myrtilli</i> (Kaltenbach)	16	Present paper [Bulgaria]
<i>Protopulvinaria pyriformis</i> (Cockerell)	16	Gavrilov, 2007 and present paper [France]
<i>Pseudopulvinaria</i> sp.	18	Moharana, 1990 [India]
<i>Pulvinaria hydrangeae</i> Steinweden	16	Nur, 1963 [California, USA]
<i>P. ribesiae</i> Signoret	18	Drozdovskiy, 1966 [Moscow, Russia]; Gavrilov, 2005 (as <i>P. vitis</i>) [St.Petersburg, Russia]
<i>P. vitis</i> (Linnaeus)	16	Drozdovskiy, 1966 [Moscow, Russia]; present paper [France]
<i>Pulvinaria</i> sp.	16	Moharana, 1990 [India]
<i>Rhizopulvinaria variabilis</i> Borchsenius	28	Gavrilov, 2007 and present paper [Astrakhan, Russia]

vinariini have been karyotyped: *Pulvinaria hydrangeae* Steinweden, 1946, *P. vitis* (Linnaeus, 1758), *P. ribesiae* Signoret, 1873, *Chloropulvinaria polygonata* (Cockerell, 1907), and *Ch. psidii* (Maskell, 1892) (Nur, 1963; Drozdovskiy, 1966; Moharana, 1990), while only *P. hydrangeae* has been studied in detail (Nur, 1963).

In this paper we present new data for 7 species from 7 genera. We also summarize and discuss all the data published, including those briefly noted in the catalogue by Gavrilov (2007a) (Table 1). In addition, we discuss here some species of Pulvinariini studied only in respect to their reproductive biology.

MATERIAL AND METHODS

All studied material has been collected by I. Gavrilov in different European regions in 2001-2008. The collecting data:

Acanthopulvinaria orientalis (Nasonov, 1908), K414, Russia, Astrakhan, sandy waste near Tinaki Lake, on twigs of *Calligonum* spp., young females, beginning of oviposition, 15.V.2004.

Protopulvinaria pyriformis (Cockerell, 1894), K531, France, Montpellier, on leaf of *Laurus nobilis*, 16.V. 2007. K 553, Portugal, Oeiras, on leaf of an unidentified tree, 26.IX.2007.

Chloropulvinaria aurantii (Cockerell, 1896), K329, Russia, Krasnodar Krai, Sochi, Khosta, on leaf of an unidentified tree, 10.V.2003. K349, Russia, Krasnodar Krai, Sochi, Khosta, on leaf of *Hedera colchica*, 5.VI.2003.

Ch. floccifera (Westwood, 1870), K340, Russia, Krasnodar Krai, Sochi, on thin twigs and needles of *Abies* sp., 26.V.2003. K456, the Ukraine, the Crimea, on needles of *Abies* sp., 30.V.2005. K 535, France, 60 km S of Paris, Fontainebleau forest, on twigs of *Ilex*, 25.V.2007. K 538, France, Paris, square near Notre Dame de Paris, on leaf of *Tilia platyphyllos*, 07.VI.2007.

Rhizopulvinaria variabilis Borchsenius, 1952, K400, Russia, Astrakhan, sandy desert near Tinaki Lake, on rhizome of *Kochia prostrata*, 10.V.2004. K411, the same locality, but on roots of *Artemisia austriaca*, 15.V.2004.

Phyllostroma myrtilli (Kaltenbach, 1874), K577-B, Bulgaria, coniferous mountain forest near resort Borovets, 1200 m above sea level, on *Vaccinium myrtillus*, 10.VII.2008.

Pulvinaria ribesiae Signoret, 1873, K280, Russia, St. Petersburg, park Sosnovka, on stem of *Betula pendula*, 19.05.2002.

P. vitis (Linnaeus, 1758), K539, France, Paris, Jardin de Plants of Museum d'Histoire Naturelle, on *Cornus* sp., 18.IV.2007. K559, France, Paris, Jardin de Luxembourg, on *Aesculus* sp., 27.IV.2008.

Eupulvinaria peregrina Borchsenius, 1953, K337, Russia, Krasnodar Krai, Sochi, on leaf of an unidentified tree, 17.05.2003. K 341, Krasnodar Krai, Adler, on leaf of *Acer mono*, 28.V.2003.

Adult females with ovisacs were fixed in aceto-ethanol (1: 3) during 24 hours, transferred to 96% ethanol and preserved in refrigerator. The gravid females were dissected under stereoscopic microscope, and the young

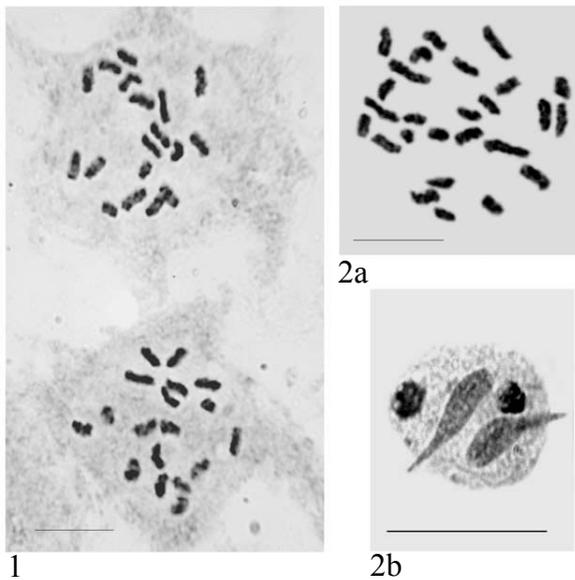
embryos were squashed in a drop of 45% acetic acid. The preparations were routinely stained using the Feulgen-Giemsa technique by Grozeva and Nokkala (1996) as previously described in Gavrilov, Trapeznikova (2007).

A part of material was stained by squashing in a drop of lactoacetocein (50 ml 85 % lactic acid: 2 g orcein: 50 ml glacial acetic acid). The primary sex ratio was evaluated by counting young embryos in the female abdomens.

RESULTS AND DISCUSSION

Acanthopulvinaria Borchsenius, 1952

Monotype genus, distributed in deserted areas of Palaearctic. Chromosome number and reproductive biology of *A. orientalis* were discussed by Gavrilov (2007b). In the population studied here $2n=18$ (Fig. 1). As few as 7% embryos demonstrated heterochromatinization of one haploid chromosome set testifying male sex of the embryos (see reviews of scale insect genetic systems: Nur, 1980; Kuznetsova, Gavrilov, 2005; Gavrilov, 2007a). Unfortunately, it is unclear, if these embryos are the progeny of one or several females, because the species is known to form dense colonies of merged ovisacs. Usually, a small proportion of male embryos is characteristic of deuterotokous coccids, and the adult males are sterile in this case (Nur, 1980). However, the anatomical study of reproductive system of 5 females (Gavrilov, 2007b) has shown that those had sperm bands in their spermathecae, so they reproduced bisexually. This unexpected result allows to formulate several hypotheses on the reproduction of *A. orientalis*. 1. Owing to certain physiological mechanisms fertilized females change the sex ratio of the progeny to the domination of female embryos. Biochemical aspects of this unique mechanism are unclear. An opinion on the male-killing ef-



Figs 1-2. 1 - *Acanthopulvinaria orientalis*, $2n=18$, female karyotype. 2, a, b. - *Chloropulvinaria aurantii*, $2n=26$. a – female karyotype. b - spermatid. Bar=10 μ m.

fect of symbiotic microorganisms is currently discussed as well (see Normark, 2004). 2. A small number of male embryos is the result of young age of studied females, then, the number of male embryos increases in older females. Examples of such dependence are known in some specially studied species of scale insects (Nur, 1990). 3. Sex ratio differs in different generations. Archangelskaya (1937) has presumed that this species is bivoltine, and this hypothesis is confirmed by the collecting material deposited in the Zoological institute Russian Academy of Sciences (oviposited females collected in July-August in different years in different localities). A summer generation of females probably produces much more songs compared to the spring one and, then, all or most part of females of the next generation appear fertilized. Definitive conclusion on the reproduction of *A. orientalis* will be possible only after further studies.

***Chloropulvinaria* Borchsenius, 1952**

According to Borchsenius (1952), the genus comprises 7 species, widely distributed in tropical and subtropical zones of the world. Chromosome numbers accompanied with idiograms of *Ch. polygonata* and *Ch. psidii* were reported by Moharana (1990) as $2n=18$ and $2n=14$, respectively. Two more species, widely distributed in Mediterranean zone, have been studied by present authors and are discussed below.

Ch. aurantii. $2n=26$ (Fig. 2, a) was discovered in the Caucasian population of the species. The oviposition takes place during cleavage divisions. Both sexes are present, but the sex ratio was not evaluated because of small number of embryos in the fixed material. The 3d-instar male larvae demonstrated last stages of spermatogenesis very similar to those in the Lecanoid system of mealybugs. The nuclei with maternal chromosomes produce sperm, while the nuclei with paternal chromosomes degenerate (Fig. 2, b).

Ch. floccifera. $2n=16$ (not shown). The embryos invariably contain a lot of lipid-like inclusions making the chromosome slide preparation difficult. There were no males and male embryos in the studied populations, then, we suggest thelytokous reproduction for this species. Gogiberidze (1938) noted male nymphs in Georgia. In a Slovak population, Řeháček (1960) figured a male wax cover without any comments on the sex ratio. Both authors probably observed occasional males reproducing parthenogenetically as in other better studied species of Pulvinariini.

***Eupulvinaria* Borchsenius, 1953**

The genus is very similar to *Pulvinaria* Targioni Tozzetti, 1866 and differs mainly in the form of ovisac and dorsal conical setae. According to Danzig (1980), these characters are not sufficient to consider *Eupulvinaria* as a genus, then, she considers it as a subgenus. In

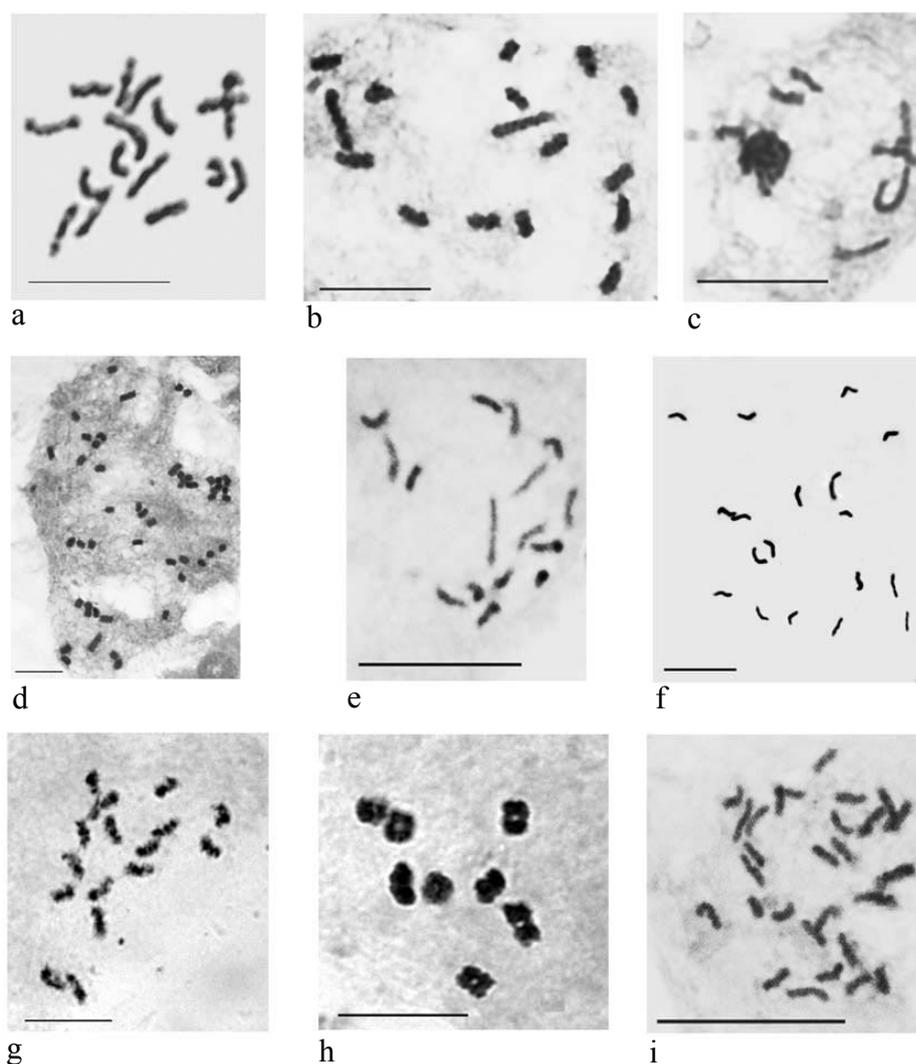


Fig. 3, a-i. Chromosomes of Pulvinariini. **a** - *Eupulvinaria peregrina*, $2n=16$; female karyotype. **b-d** - *Phyllostroma myrtilli*, $2n=16$; **b** - female karyotype, **c** - male, heterochromatinization of paternal set of chromosomes, **d** - cell of embryonal polyploid sector, $6n=48$. **e** - *Pulvinaria vitis*, $2n=16$; female karyotype. **f** - *P. ribesiae*, $2n=18$; female karyotype. **g-h** - *Protopulvinaria pyriformis*, $2n=16$; **g** - female karyotype; **h** - meiotic metaphase I. **i** - *Rhipozpulvinaria variabilis*, $2n=28$; female karyotype. Bar=10 μm .

the present paper we prefer using the Borchsenius' (1952, 1957) conception, because the genus *Pulvinaria* is rather large (about 140 species), then, it is more convenient to consider some species groups of *Pulvinaria* sensu lato as separate genera. The genus *Eupulvinaria* comprises 8 species, 5 of which have subtropical Indo-Malasian distribution, two inhabit Far East of Russia and one species inhabits

European subtropics of Caucasus.

E. peregrina, $2n=16$ (Fig. 3, a), is the only cytogenetically studied species of the genus. The oviposition takes place during cleavage divisions. Probably obligate thelytokous species. Males have never been found, and the embryos with heterochromatinization were absent in ovisacs of the studied population (Sochi, Black Sea coast of Russian Caucasus).

***Neopulvinaria* Hadzibejli, 1955**

Monotype genus with a species *N. innumerabilis* (Rathvon, 1854), distributed in Canada, USA and Caucasus. There are no cytogenetical data, but Nur (1980) gives a very brief note on deuterotokous and diploid arrenotokous reproduction of the species.

***Phyllostroma* Šulc, 1942.**

Monotype genus, having European distribution. *Ph. myrtilli* displays $2n=16$ (Fig. 3, b, c). There were a lot of adult males in the studied population; primary sex ratio was 36 % males: 64 % females. However, spermathecae and oviducts of 5 females studied had no sperms. Thus, females as well as males of *Ph. myrtilli* are probably produced by parthenogenesis and deuterotoky. Polyploid cells, most likely those of mycetome-bacteriome, as in mealybugs and armored scales, were found in embryos (Fig. 3, d).

***Pulvinaria* Targioni Tozzetti, 1866**

The largest genus of Pulvinariini with about 140 species, widely distributed in the tropical and the temperate zones of the world.

P. hydrangeae Steinweden, 1946. The evidence on cytogenetics and reproductive biology of this species is based on the comprehensive study by Nur (1963). $2n=16$. Thelytokous reproduction has been revealed in 3 studied USA populations. Sperms have never been observed in spermathecae of all studied females and inside the eggs. In two populations (with thousands studied embryos from different females), not a single embryo had paternal heterochromatinization of haploid chromosome set. In the third population, females had from 0 till 60 % embryos with heterochromatinization and up to 90 % degenerating embryos, which probably were parthenogenetically produced as well.

P. ribesiae and *P. vitis* have small morphological differences and are considered by the

majority of authors as synonyms. However, Borchsenius (1957) and Drozdovskiy (1966) have considered them as separate species on the base of the body size, phenological and ecological differences. Drozdovskiy has also reported different chromosome numbers, $2n=18$ and $2n=16$, respectively, for these species. According to him, *P. ribesiae* has smaller size, overwinters as larva and lays eggs in June-July, whereas *P. vitis* has bigger size, overwinters as adult female and lays eggs in May. This information is well supported by the observations of Evelyn Danzig (personal communication). Our chromosome study also agrees with the data of Drozdovskiy. We have studied a population comprising small females with $2n=18$ and populations comprising large females with $2n=16$ (Fig. 3, e, f). Chris Malumphy (Malumphy, 1991) in his PhD thesis has also noted $2n=16$ for English populations of *P. vitis*.

As for *Pulvinaria regalis* Canard, 1968, **syn. n.**, we are placing this nominal species in synonymy under *Pulvinaria vitis* Linnaeus, 1758. There are no morphological differences between the type series of *P. regalis*, studied by the first author in Paris Museum d'Histoire Naturelle and numerous series of *P. vitis* from different Palaearctic regions. There are no cytogenetical (present paper) as well as enzyme-isoenzyme banding and thin morphological (revealed under scanning electron microscope) differences (Malumphy, 1991).

***Pulvinariella* Borchsenius, 1953**

The monotype genus with a species *P. mesembryanthemi* (Vallot, 1829) widely distributed in Mediterranean zone, Australasia, more locally in Afrotropical zone and also in California (USA) and Argentina. There are no cytogenetic data for this species, but some information on reproductive biology can be found in Pesson (1941). This author has supposed

that the species reproduces parthenogenetically, by deuterotoky. The male nymphs appear in groups on host plants, and each group is probably the offspring of a single mother. Mature males ignored females, which had no sperms in spermathecae. According to Nur (in the review of 1982), the species demonstrates some mosaic embryos with and without a heterochromatic set of chromosomes.

***Protopulvinaria* Cockerell, 1894**

The genus includes 3 species, two tropical and *P. pyriformis*, distributed also in subtropical regions of Europe and America. Only *P. pyriformis* is studied cytogenetically (Gavrillov, 2007a; present study). $2n=16$ (Fig. 3, g). Oviposition takes place during invagination of the germ band in an embryo. Up to 40 embryos were found inside the mother's body. There have been no males in studied populations. About 140 embryos from 14 females were studied and no one had heterochromatinization of the paternal set of chromosomes. One embryo with 5 cells only demonstrated a slightly more condensation of one haploid set of chromosomes. In 5 studied females, spermathecae had no sperms. This species is probably obligatorily parthenogenetic with meiotic thelytoky. Fig. 3, h shows an oogonial metaphase with 8 bivalents displaying terminal chiasmata. The way of the diploidy restoration remained unclear.

***Rhizopulvinaria* Borchsenius, 1952**

This genus currently includes 32 nominal species, mainly distributed in desert or semi desert zones of Palaearctic. After a revision of the group (preparing by the first author) many of these species will probably be synonymized, because there is a very significant individual and interpopulational variation in the genus, and many described species are probably the local morphs. This variation may be related to the obligate thelytoky, which is probably pres-

ent in *Rhizopulvinaria* spp.

Rhizopulvinaria variabilis. $2n=28$ (Fig. 3, i). It is the highest chromosome number in the Pulvinariini studied till now. The oviposition takes place just after cleavage divisions. This species seems to have obligate thelytoky. Males have never been found, and embryos with heterochromatinization are absent in ovi-sacs of the studied population.

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