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Karyotypes of three species of the genus *Trissolcus* Ashmead, 1893 (Hymenoptera: Scelionidae)

Кариотипы трех видов рода *Trissolcus* Ashmead, 1893 (Hymenoptera: Scelionidae)

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КЛЮЧЕВЫЕ СЛОВА. Telenominae, кариотип, морфометрия хромосом.

ABSTRACT. Karyotypes of three species of the genus *Trissolcus* Ashmead, 1893, namely, *T. kozlovi* Rjachovsky, 1975, *T. rufiventris* (Mayr, 1907) and *T. semistriatus* (Nees von Esenbeck, 1834), were studied for the first time. Although all examined members of the genus appeared to have 2n = 20, chromosome morphometrics revealed substantial differences between karyotypes of certain species.

РЕЗЮМЕ. Впервые изучены кариотипы трёх видов рода *Trissolcus* Ashmead, 1893: *T. kozlovi* Rjachovsky, 1975, *T. rufiventris* (Mayr, 1907) и *T. semistriatus* (Nees von Esenbeck, 1834). Хотя у всех исследованных представителей рода обнаружено 2n = 20, морфометрия хромосом выявила существенные различия между кариотипами некоторых видов.

Introduction

Parasitoid Hymenoptera is one of the most species-rich, taxonomically complicated and economically important insect groups [Godfray, 1994; Quicke, 1997], with its estimated species number in the world fauna exceeding one million [Bebber *et al*., 2014; Forbes *et al*., 2018]. Despite rapid accumulation of karyotypic data, they are still available for just about 500 parasitoid species [Gokhman, 2009]. The superfamily Platygastroidea currently comprises more than 6000 described species [Platygastroidea, 2019]. This group is traditionally subdivided into two separate families, Platygastridae and Scelionidae; however, they are often lumped into Platygastridae s.l. (see discussion in [Popovici et al., 2017]). Among Scelionidae, karyotypes of just three members of the subfamily Telenominae were previously studied [Dreyfus, Breuer, 1944; Gokhman, 2009; Fusu *et al*., 2013]. Although all examined species appeared to have the same haploid chromosome number, n = 10, the known data on chromosome morphology suggest that at least some members of the family can differ in this respect [Dreyfus, Breuer, 1944; Fusu *et al*., 2013]. We have recently studied karyotypes of three species of Telenominae that belong to the genus *Trissolcus* Ashmead, 1893. The results of this work are given below.

Material and methods

Origin of parasitoids

Parasitic wasps used in the present study were reared from egg clutches of true bugs (Hemiptera: Pentatomidae) or collected by sweeping in their natural habitats in a few regions of European Russia, namely, in the Moscow and Volgograd Provinces as well as in the Republic of Kalmykia, in 2014-2016 (Table 1). Specimens of *Trissolcus kozlovi* Rjachovsky, 1975 were reared from parasitized eggs of the green shield bug, *Palomena prasina* (Linnaeus, 1761), and then transferred in the lab to another host, *Graphosoma lineatum* Linnaeus, 1758. Individuals of *T. rufiventris* (Mayr, 1907) were collected alive and then temporarily cultivated in the lab on *G. lineatum*. Egg clutches of the same host were deposited in the field to collect *T. semistriatus* (Nees von Esenbeck, 1834). Fresh egg clutches of *G. lineatum* were given to the fertilized parasitoid females for oviposition. To obtain wasp prepupae, parasitized host eggs were incubated in lab conditions for a few days.

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Table 1. Species of the genus *Trissolcus* used in the present study.
Таблица 1. Виды рода *Trissolcus*, использованные в настоящей работе.

Species	Locality	No. studied specimens	No. studied (haploid) and diploid metaphase plates
T. kozlovi Rjachovsky, 1975	Prioksko-Terrasny Natural Reserve, about 100 km S Moscow	2	12
T. rufiventris (Mayr, 1907)	NW Mikhailovka, about 220 km NW Volgograd	3	(7) 2
	S Frolovo, about 110 km NW Volgograd	1	1
T. semistriatus (Nees von Esenbeck, 1834)	Razdory, about 20 km W Moscow	2	7
	NW Dubovka, about 50 km N Volgograd	4	17
	E Malye Derbety, about 190 km NE Elista	3	11

Species used in the present study were identified by Elijah Talamas and Alexander Timokhov. Voucher adult specimens of parasitoids are deposited in the collection of the Department of Entomology of Moscow State University (Moscow, Russia), and in the Florida State Collection of Arthropods (Gainesville, Florida, USA).

Preparation of chromosomes

Chromosome preparations were made from cerebral ganglia of parasitoid prepupae using a modified version of the technique described by Imai et al. [1988]. Wasps were dissected in 0.5% hypotonic sodium citrate solution containing 0.005% colchicine, and the tissues were incubated in fresh solution for 30 minutes at room temperature. The material was transferred to a pre-cleaned microscope slide using a Pasteur pipette and gently flushed with Fixative I (glacial acetic acid: absolute ethanol: distilled water 3:3:4). Tissues were disrupted in an additional drop of Fixative I using dissecting needles. Another drop of Fixative II (glacial acetic acid: absolute ethanol 1:1) was then applied to the center of the area and blotted off the edges of the slide. The slide was air dried at room temperature. Preparations were stained with freshly prepared 3% Giemsa solution in 0.05M Sorensen's phosphate buffer (Na_2HPO_4 + KH_2PO_4, pH 6.8).

Table 2. Relative lengths of chromosomes of three species of the genus *Trissolcus* (mean ± SD).
Таблица 2. Относительная длина хромосом трёх видов рода *Trissolcus* (среднее значение ± стандартное отклонение).

Chromosome no.	*T. kozlovi*	*T. rufiventris*	*T. semistriatus*
1	15.96 ± 0.52	16.04 ± 0.95	14.49 ± 0.79
2	12.07 ± 0.02	14.40 ± 1.43	12.39 ± 1.02
3	10.56 ± 0.07	12.44 ± 0.71	11.03 ± 0.58
4	10.22 ± 0.26	11.43 ± 0.16	10.43 ± 0.36
5	9.70 ± 0.18	10.20 ± 0.81	9.93 ± 0.44
6	9.49 ± 0.03	8.84 ± 0.80	9.31 ± 0.41
7	9.27 ± 0.03	7.82 ± 0.06	8.77 ± 0.17
8	8.90 ± 0.45	6.79 ± 0.40	8.41 ± 0.22
9	7.68 ± 0.25	6.32 ± 0.57	8.02 ± 0.29
10	6.15 ± 0.34	5.72 ± 0.93	7.22 ± 0.46

Image acquisition and analysis

Mitotic divisions were studied and photographed using an optic microscope Zeiss Axioskop 40 FL fitted with a digital camera AxioCam MRc (Carl Zeiss, Oberkochen, Germany) (Table 1). To obtain karyograms, the resulting images were processed with image analysis programs Zeiss AxioVision version 3.1 and Adobe Photoshop version 8.0. Chromosomes from selected diploid metaphase plates were measured using KaryoType software version 2.0 (Table 2). Since centromere positions could not be reliably identified for many chromosomes, only average values of relative lengths of chromosomes (RLs) are given in the present paper. Nevertheless, certain chromosomes were classified using results from selected metaphase plates.

Results and discussion

Trissolcus kozlovi (Fig. 1). 2n = 20. Chromosomes more or less gradually decrease in size, but the first pair is obviously longer than the remaining elements (Table 2). At least some chromosomes are clearly bi-armed.

T. rufiventris (Fig. 2). n = 10 and 2n = 20. In this species, as in the previous one, chromosomes can be arranged in a more or less continuously decreasing order of size. The first and second chromosome pairs are slightly longer than the remaining elements. As in *T. kozlovi*, at least some chromosomes are obviously bi-armed.

Figs 1–3. Diploid karyograms of *Trissolcus* species: 1 — *T. kozlovi*, 2 — *T. rufiventris*, 3 — *T. semistriatus*. Bar = 10 µm.
Рис. 1–3. Диплоидные кариограммы видов *Trissolcus*: 1 — *T. kozlovi*, 2 — *T. rufiventris*, 3 — *T. semistriatus*. Масштаб 10 µm.

T. semistriatus (Fig. 3). 2n = 20. As in the two previous species, chromosomes more or less gradually decrease in size. The first chromosome pair is slightly longer than the remaining elements. Again, at least some chromosomes (e.g. those of the first two pairs) are clearly bi-armed. No karyotypic variation was detected between specimens of *T. semistriatus* having different geographic origin.

Previously, Dreyfus & Breuer [1944], Gokhman [2009] and Fusu *et al*. [2013] examined chromosome sets of three species of Scelionidae of the subfamily Telenominae, i.e. *Telenomus fariai* Costa Lima, 1927, *T. turesis* Walker, 1836 [= *T. chloropus* (Thomson, 1861)] and *Trissolcus basalis* (Wollaston, 1858) respectively. Nevertheless, our paper represents the first comparative karyotypic study of the genus *Trissolcus*. Nowadays, chromosomes of only four members of the genus *Trissolcus* — *T. basalis*, *T. kozlovi*, *T. rufiventris* and *T. semistriatus*, are known (see Fusu *et al*. [2013]). Although all these species have the same chromosome number, 2n = 20, they belong to different species groups [Talamas *et al*., 2017]. Specifically, *T. kozlovi* is included into the *flavipes* group, while the three other species represent the *basalis* group. Our study demonstrates that all studied members of *Trissolcus* differ in some details of their karyotype structure as well (see Table 2). For example, *T. basalis* is perhaps the closest to the hypothetical ancestor of the Cynipoidea + Proctotrupoidea s.l. + Chalcidoidea clade in terms of its karyotype structure [Gokhman, 2011]. The ancestral haploid chromosome set is supposed to contain eleven acrocentrics (or subtelocentrics), and the karyotype similar to that of *T. basalis* could therefore originate via central chromosomal fusion (see Gokhman [2009]). On the other hand, the karyotype of *T. kozlovi* resembles that of *T. basalis* by having the first chromosome pair substantially longer than the remaining elements. However, this feature is an apparent symplesiomorphy of the two species, and subsequent rearrangements could alter the karyotype structure of the other members of the genus.

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