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Contribution to Karyological Data of Terrestrial Mollusks (Mollusca: Gastropoda) of Georgia

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ABSTRACT. The results of the karyological investigations of the terrestrial mollusks of Georgia (Caucasus region) are given. The samples from western Georgia (Imereti and Samegrelo-Zemo Svaneti regions) were used. The basic chromosome numbers of four species belonging to families: Clausiliidae, Hygromiidae, Oleacinidae and Pomatiidae were established. In clausiliid species *Elia (Megaleuxina) derasa* basic chromosome number $n=27$ was registered, for hygromiid snail *Fruticocampylaea narzanensis* $n=23$, $n=26$ was recorded in oleacinid *Poiretia mingrelica* and $n=13$ in pomatiid species *Pomatias rivularis*. The chromosome numbers registered in the investigated species of Georgian land snails: *E. derasa*, *F. narzanensis*, *P. mingrelica* and *P. rivularis* are within the ranges of chromosome numbers described for families Clausiliidae, Hygromiidae, Oleacinidae and Pomatiidae, respectively. $n=27$ is the new chromosome number in the family Clausiliidae. The numbers $n=23$, $n=26$, $n=27$ are basic chromosome numbers characteristic of Caucasian and Georgian terrestrial mollusks. © 2018 Bull. Georg. Natl. Acad. Sci.

Key words: chromosome numbers, terrestrial mollusks, Clausiliidae, Hygromiidae, Oleacinidae, Pomatiidae, Georgia

Terrestrial malacofauna of Georgia is very rich (more than 250 species) among which over 25% of species are Georgian and around 65% are Caucasian endemics [1]. Until recently studies of Georgian terrestrial mollusks were concentrated only on taxonomy and biodiversity inventory while cytogenetic studies were completely lacking. The results of the first karyological investigations of Georgian mollusk species are given in our several publications [2, 3]. Here, we provide an additional karyological data on other terrestrial mollusk

species of Georgia to contribute karyological knowledge in this diverse animal group.

Materials and Methods

In April-May 2015 and 2017 the samples were collected in western Georgia: Imereti (villages: Dzedzileti, Gordi and Katskhi, river Chishura) and Samegrelo-Zemo Svaneti (villages: Gachedili and Salkhino) regions. In total 126 specimens of four species were collected. Living specimens were kept in plastic containers with damp substrate (at 180 C temperatures) for several months until the

Table 1. Summary table on species examined, locality data and chromosome numbers

Species	Locality name	Altitude (m)	Longitude	Latitude	Specimens/plates	Chromosome numbers (n)
<i>E. derasa</i> (Clausiliidae)	Salkhino	250	42.4939	42.345	11/30	27
	Gordi	600	42.4561	42.5314		
<i>F. narzanensis</i> (Hygromiidae)	Gachedili	240	42.4714	42.375	2/62	23
<i>P. mingrelica</i> (Oleacinidae)	Katskhi	592	42.2856	43.2158	2/51	26
	Katskhi	610	42.2844	43.2164		
<i>P. rivularis</i> (Pomatiidae)	River Chishura	113	42.2164	42.7956	10/69	13
	Dzedzileti	420	42.4119	42.5703		

treatment. Chromosome slides were prepared from gonad tissues using air-drying and squash methods [2]. The animals were injected with 0.05% colchicine for 20 to 23 h before dissection. Gonads were cut into small pieces, subjected to hypotonic treatment for 60 min in distilled water and then centrifuged at 1000 rpm for 10 min. The supernatant was replaced with fresh mixture of 96% ethanol and glacial acetic acid (3:1). This procedure was repeated three times. The cell suspension was dropped on clean, cold and damp glass slides. The slides were air-dried. Chromosome preparations of small mollusks were made following the squash method. Specimens were immersed for 2 h in a 0.01% freshwater colchicine solution at room temperature. Gonads were extracted from specimens and transferred into a KCl 0.075 M hypotonic solution for 20 min. They were fixed in freshly prepared 60% acetic acid solution for 5 min and squashed between the glass slide and coverslip.

The prepared chromosome slides were stained with a Romanovskii solution of azur-eosine (pH 6, 8). The slides were observed under a PH 100D microscope (at 10×100 magnification). The well-spread chromosome plates were selected for analysis, counting of chromosomes and subsequent photography (PHMIAS version 2.0).

The studied species, localities, number of specimens that were suitable for karyological analysis and chromosome number data are given in Table 1.

Results and Discussion

Only meiotic stages (pachitene, diplotene, diakinetik and metaphase I) were observed in investigated species of families Clausiliidae Gray, 1855, Hygromiidae Tryon, 1866, Oleacinidae H. Adams & A. Adams, 1855 and Pomatiidae Newton, 1891. The mitotic chromosomes were not observed in them. As some authors mark, literature on karyotype analysis of molluscs is very poor due to difficulties of obtaining mitotic plates [4, 5]. According to Boato, the gonial cells are in mitosis for very short time periods [6].

The modal haploid chromosome numbers (n=) 27, 23, 26 and 13 were determined in meiotic plates of the species studied: *Elia derasa* (Mousson, 1863), *Fruticocampylaea narzanensis* (Krynicky, 1836), *Poiretia mingrelica* (O. Boettger, 1881) and *Pomatias rivularis* (Eichwald, 1829), respectively (Figs. 1, 2).

The species *F. narzanensis*, *P. mingrelica* and *P. rivularis* with chromosome numbers (n=) 23, 26 and 13, respectively, do not show any significant deviation in chromosome numbers known for other species from the respective families [2, 7-11]. These numbers are within the ranges of basic chromosome numbers described for Hygromiidae, Oleacinidae and Pomatiidae [10, 12]. The species *F. narzanensis* and *P. mingrelica* are endemic land snails of Caucasus and Georgia, respectively. The numbers n=23 and n=26 which are found in these species, are basic chromosome numbers characteristic of Caucasian and Georgian terrestrial mollusks.

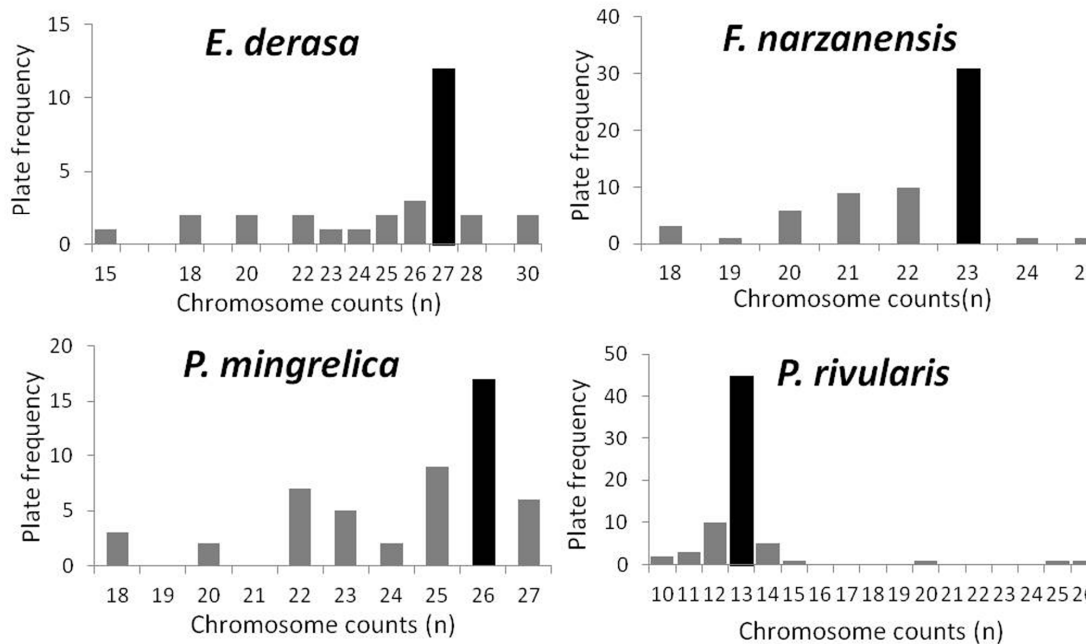


Fig. 1. The results of chromosome counting in species studied.

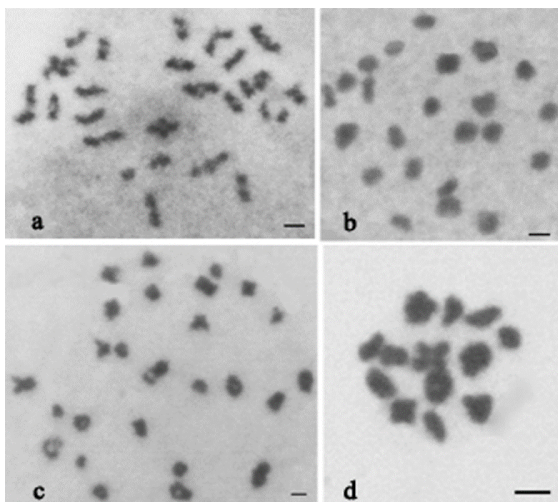


Fig. 2. Meiotic chromosome spreads of species studied. *E. derasa* (a); *F. narzanensis* (b); *P. mingrelica* (c) and *P. rivularis* (d). Scale bar = 5 μ m.

Clausiliid snail *E. derasa* possess chromosome number $n=27$ that is a new number not reported until now in Clausiliidae. More than 1300 species of the family Clausiliidae are known worldwide

[13], out of which some 33 species from Europe and Asia are investigated karyologically. The basic chromosome numbers within this family was reported as ($n=$) 24, 28, 30 and 31 with domination of 24 [4, 7, 14, 15]. From the Caucasus, at least 54 species of Clausiliidae are known (49 endemic) with no karyological data until now [16]. Observed chromosome number $n=27$ in *E. derasa* possibly is the new basic chromosome number in the family Clausiliidae. From this point of view, the investigation of other species of Georgian clausiliids is necessary. *E. derasa* is endemic snail of Caucasus. $n=27$ which is found in this species, is basic chromosome number characteristic of Caucasian terrestrial mollusks.

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ზოოლოგია

საქართველოს ხმელეთის მოლუსკების (Mollusca: Gastropoda) კარიოლოგიური მონაცემები

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მოცემულია საქართველოს (კავკასიის რეგიონი) ხმელეთის მოლუსკების კარიოლოგიური გამოკვლევების შედეგები. დადგენილია ოთხი ოჯახის: Clausiliidae, Hygromiidae, Oleacinidae და Pomatiidae სახეობების ქრომოსომული რიცხვები. ოჯახ Clausiliidae-ს სახეობაში *Elia (Megaleuxina) derasa* რეგისტრირებულია ბაზალური ქრომოსომული რიცხვი $n=27$, Hygromiidae-ს სახეობაში *Fruticocampylaea narzanensis* $n=23$, $n=26$ აღინიშნება Oleacinidae-ს სახეობაში *Poiretia mingrelia*, ხოლო ოჯახ Pomatiidae-ს სახეობისთვის *Pomatias rivularis* $n=13$. ქრომოსომული რიცხვები, რომლებიც რეგისტრირებულია საქართველოს ხმელეთის მოლუსკების გამოკვლეულ სახეობებში: *E. derasa*, *F. narzanensis*, *P. mingrelia* და *P. rivularis* მოთავსებულია მსოფლიო მალაკოფაუნის Clausiliidae-ს, Hygromiidae-ს, Oleacinidae-ს და Pomatiidae-ს ოჯახებისთვის დამახასიათებელ, ქრომოსომული რიცხვების ვარიეტების დიაპაზონში. $n=27$ ახალი ქრომოსომული რიცხვია Clausiliidae-ს ოჯახისთვის. $n=23$, $n=26$ და $n=27$ კავკასიის და საქართველოს ხმელეთის მოლუსკებისთვის დამახასიათებელი ბაზალური ქრომოსომული რიცხვებია.

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