საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 16, №1, 2022
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 16, no. 1, 2022

Parasitology and Helminthology

The Case of Detecting Cestodes in Mole-Rat *Nannospalax xanthodon* in Georgia

Ketevan Nikolaishvili[*], Tsitsino Lomidze[*], Lali Murvanidze[*], Lela Arabuli[*], Ketevan Asatiani[*]

[*]*Institute of Zoology, Ilia State University, Tbilisi, Georgia*

(Presented by Academy Member Tinatin Sadunishvili)

The aim of the current study was to investigate the helminth fauna of the mole-rat, *Nannospalax xanthodon* (Rodentia, Spalacidae), inhabiting the Javakheti plateau in southern Georgia. Three mole-rats were captured at the site of Aspindza and Akhalkalaki municipalities. As a result of complete helminthological examination, only cestodes were found in the small intestine. The material was processed using the methods accepted in helminthology. All mole-rats were infested. The intensity of invasion was 1-2 specimens per animal. The morphometric study of cestodes was performed. Comparing the morphological and morphometric data of scolex, neck, proglottides and eggs of the studied helminths with the data known in the literature, we consider that cestodes, first discovered in the mole-rat *N. xanthodon* in southern Georgia, belong to the order Cyclophyllidea (Braun, 1900), family Anoplocephalidae (Cholodkowsky, 1902) and to the genus *Paranoplocephala* Lűhe, 1910.

Mole-rat, cestodes, Javakheti plateau, Georgia

Rodents represent the most abundant species-rich mammalian order adapted to life in different niches. The helminth fauna of these rodents, including mole-rats, has been studied for a long time and the interest taken in it is not waning even today [1-9].

In Georgia, the studies on the helminth fauna of rodents were started by Y.D. Kirshenblat. Investigating the regularities of the dynamics of parasitic fauna of animals, he systematically studied rodents in various areas of Georgia. The data were obtained on the species composition of helminths parasitizing in most rodent species, the distribution of their larval forms in various hosts and geographical distribution within the country. The research findings are presented in the papers [10-13]. In subsequent years, the study of the helminth fauna of rodents was continued by Matsaberidze [14]; Prokopič, Matsaberidze [15]; Kurashvili *et al* [16].

In the materials on the helminth fauna of 25 species of Georgian rodents, studied by Kirshenblat [12], the mole-rat *Spalax leucodon* from the Akhalkalaki region is also indicated. In the small intestine of these animals, only nematodes of the

genus *Ascaris sp*. were found by him. That was the first and the only record on mole-rat helminths in Georgia.

Due to the peculiar environment, mole-rats, like some specialized forms of rodents adapted to the underground lifestyle, do not differ in the diversity of the parasitic fauna, which is confirmed both by the above-mentioned work of Kirshenblat [12] and the studies of a number of authors by Tinnin [4]; Tamam [17]; Vlasov et al. [6]; Archer et al., [9]. In Georgia, the mole-rat has been poorly studied by parasitologists. In September 2018, we had an opportunity to conduct a helminthological study of these animal species rare for Georgia. Preliminary data on cestodes found in mole-rats were presented at the international conference BIO Eco 2019 in Istanbul (Turkey) [18]. In this article, we present the results of further studies on cestodes.

Material and Methods

The objective of this study was three mole-rats *Nannospalax xanthodon*, (Syn.: *N. nehringi*) [19]. The animals were captured in the south of Georgia on the Javakheti plateau in private grain fields. Among them, one female from the Aspindza municipality, Lepisi (Niala plateau) N $41^0 22' 55''$, E $43^0 12' 36''$ and two females from the Akhalkalaki municipality in the vicinity of the village Myasnikyan N $41^0 18' 11''$, E $41^0 19' 12''$, respectively, from the left and right banks of the river Mtkvari at an altitude ~1800 m a.s.l. As a result of a complete helminthological examination of the internal organs, only two scolexes and five strobili with several fragments were extracted from the small intestine of three animals. Three scolexes were not found. The isolated cestodes were washed in water. We studied both, native and fixed in 70^0 alcohol and clarified in glycerin temporary preparations. Permanent preparations were prepared by placing them in aceto-carmine, then in 70^0 acid-alcohol, washed with water, dehydrated successively by transferring them into ascending (70^0-90^0) alcohols. The preparations were fixed with Canada balsam. The morphology of cestodes was studied using a stereo microscope dual lights with 10 MP USB digital camera. To determine the taxonomic affiliation of the studied cestodes, their morphological and morphometric parameters were compared with the corresponding descriptions from literature [10, 20-22]. Measurements are given in millimeters (mm). The material is stored in the collection of the Institute of Zoology at the Ilia State University in Tbilisi.

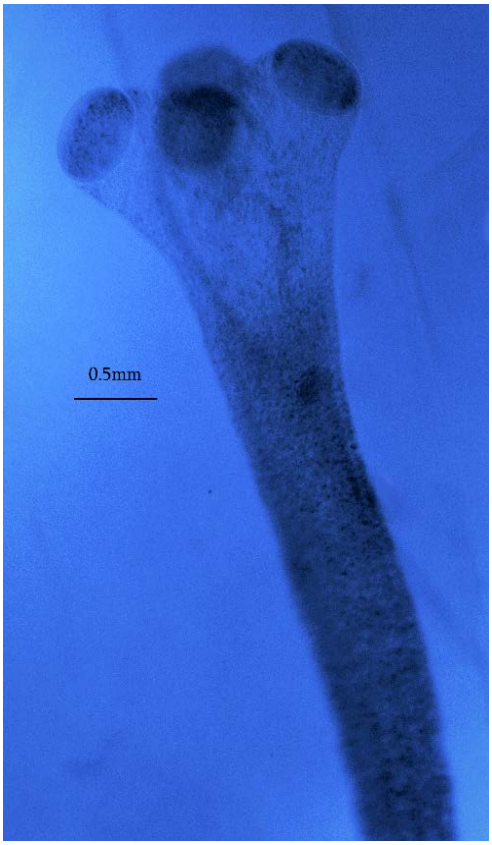

Fig. 1. *Paranoplocephala* cestode from mole-rat in Georgia (conventionally Zia-2) Scolex.

Results and Discussion

All three mole-rats *N. xanthodon* were infected only with cestodes localized in the small intestine. The intensity of invasion was 1-2 specimens per animal. The mole-rat from the Aspindza municipality and their cestodes are conditionally designated Zia-2. Strobila of Zia-2 is ribbon-like, milky white. Length 152-200 mm, at the beginning

it is narrow, and towards the posterior end, it expanded due to mature segments. The maximum width of the narrowest part of the strobila is 1.2 mm, the middle part is 3.5 mm, and the lower end is 4 mm. Mature proglottides are transversely elongated. In 1 cm length of the mature part of the strobila, which is 2 mm wide, had about 45 proglottides, and 15 cm long - 684. Scolex (Fig. 1) smooth, maximum width about 2 mm, had the shape of a funnel, equipped with four laterally located rather powerful round suckers, noticeably rising above the surface of the scolex. The latter smoothly turned into a well-defined neck. Its length is 12-40 mm, measured from the lower end of the sucker to the first indicator segment, its width is 0.66 mm. The neck was followed by a monotonous row of proglottides with an unformed reproductive system. In the middle part of the strobili were hermaphroditic proglottides. They had a weak craspedote. The genital pores are unilateral, their location is almost equatorial. In the area of mature proglottids in Zia-2, protruding unarmed cirrus were clearly visible (Fig. 2). They were observed in one section of the strobila in a row in five proglottids, and in another section in a row in nine proglottids. The length of the last section of the strobila is 1.5 mm, and the width is 0.8 mm. Mature female gonads were located symmetrically on both sides of the hermaphroditic proglottides. In the proglottides of the posterior end of the strobila, the entire space was occupied by eggs. Zia-2 eggs are mostly oval in shape, covered with a thin, smooth, transparent shell with 1-2 dark stripes. Eggs were found at different stages of larval development. The following were visible: an early stage, a developing larva with an orientation towards the pole, towards the dark stripe of the egg, and an already formed cysticercoid. Egg size varied from 0.0505 x 0.027 mm to 0.0574 x 0.0366 mm (n=7). The dimension of the isolated Zia-2 egg with embryo was 0.0573 x 0.0314 mm.

Two mole-rats from Akhalkalaki municipality and three strobilae without scolex isolated from them were designated Mia-4 and Mia-5. These cestodes, like Zia-2, milky-white in color, also had a ribbon-like body, but their length was longer and amounted to 200-240 mm. The immature proglottides square in shape, while sexually mature proglottides elongated in the longitudinal direction. Pregravid proglottides had a branched uterus. As the eggs mature, the proglottides noticeably increase in length (Fig. 3). Mia-4 and Mia-5 eggs round, covered with a smooth, thin, transparent shell. Eggs diameter ranged from 0.0381 to 0.0452 mm (n=4). They were at different stages of development of eggs Mia-4 and Mia-5. We suppose that the morphological differences found between the cestodes Zia-2 and Mia-4 and Mia-5 are associated with the ecological and geographical (Mtkvari River) dissociation of mole rats, which excludes their meeting. However, it should be mentioned that the chromosomal form of all *N. xanthodon* was the same 2N=50 [23].

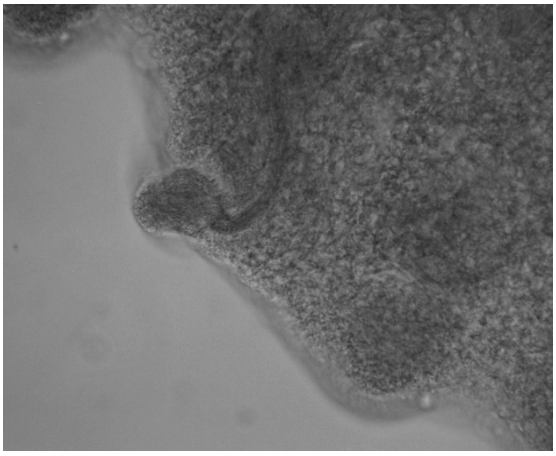

Fig. 2. *Paranoplocephala* cestode from mole-rat in Georgia (conventionally Zia-2) Mature proglottids with cirrus.

There is little information in the literature on mole-rat cestodes. In the small intestine of the mole-rat *Spalax leucodon* Nordmann from Moldova, a cestode of the genus *Aprostatandrya* sp. was found by Andreiko [1]. Later, when discussing the helminth fauna of the mole-rats of the fauna of the USSR, Andreiko [2] mentions *Aprostatandrya caucasica* from valid cestodes,

which, according to the Fauna Europaea website (http://www.fauna-eu.org/) Syn. *Andrya caucasica* (Kirshenblatt, 1938), *Paranoplocephala caucasica* (Kirshenblatt, 1938). Fair *et al.* [3] described a new species of cestode *Paranoplocephala nevoi* n.sp. from Palestine mole-rat *Spalax ehrenbergi* Nehring, 1898 with chromosome form 2N=54 (Wertheim, Nevo, 1971) [24]. Separate morphological data of this cestode, such as the shape of a scolex, a well-defined neck, transversely elongated fertilized proglottides, unilateral genital pores, a small cirrus, have some similarities with data from Zia-2 cestode, and the round shape of the eggs resembled eggs of cestodes in *N. xanthodon* Mia-4, Mia 5. However, there were significant differences in size and in some external morphological parameters between the compared parasites and their hosts. This fact can be considered one of the manifestations of the adaptive relationship between the parasite and the host.

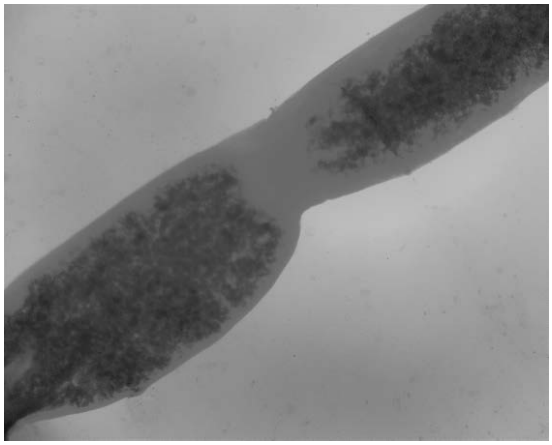

Fig. 3. *Paranoplocephala* cestode from mole-rat in Georgia (conventionally Mia-4 and Mia-5) Gravid proglottids.

In order to establish and clarify the taxonomic status of cestodes of genus *Paranoplocephala* from different rodent species, a number of authors re-describe them on the basis of a comparison of morphological characters. Thus, Rausch [20] synonymized the cestode of the genus analysis *Aprostatandrya* (Kirshenblat, 1938) from voles into the genus *Paranoplocephala* Lűhe, 1910. As a result of a taxonomic revision of cestodes from voles, squirrels, and shrews, Haukisalmi and Henttonen [25, 26] concluded that one of the factors determining the status of a species is host specificity and its geographical distribution. It should also be noted the work of Haukisalmi et al., [27] which presents an extensive phylogenetic analysis and revision of paranoplocephalid-like cestodes. The analysis was based on a sequential study of the DNA of two parts of the mitochondrial genes. Based on the phylogenetic relationship and the main morphological features of particular importance, the authors in a strict order identified twelve new genera attributed to *Paranoplocephala*.

Comparing the results of our research with the above literature data, we believe that cestodes, first found in the mole-rat *N. xanthodon*, living in southern Georgia, belong to the order Cyclo-phyllidea (Braun, 1900), family Anoplocephalidae (Cholodcowsky, 1902) and can be attributed to the genus *Paranoplocephala.* We hope that the application of the molecular genetic techniques in the future will confirm that the helminths studied by us belong to the already known genera of paranoplocephalid-like (*Paranoplocephala* Lűhe, 1910-like) cestodes and make it possible to establish their generic and species specificity to their host *N. xanthodon* from the Aspindza and Akhalkalaki municipalities of the southern region of Georgia.

Conclusion

The mole-rat *N. xanthodon* living in the south of Georgia is a specific animal for the given area. Due to the underground way of life, it is protected from infection by a wide range of various invasive sources, which explains the lack of diversity of its helminth fauna. The initial studies in Georgia concerned only the detection of nematodes in mole-rats [12]. The researched mole-rats *N. xanthodon* were infested only with cestodes. Based on a

number of morphometric features, the cestodes of the mole-rat *N. xanthodon* are assigned to the order Cyclophyllidea (Braun, 1900), family Anoplocephalidae (Cholodkowsky, 1902) and to the genus *Paranoplocephala*. The present work could supplement the materials on the helminth fauna of the mole-rats and micromammals of Georgia.

პარაზიტოლოგია და ჰელმინთოლოგია

ბრუცა *Nannospalax xanthodon*-ის ცესტოდებით ინვაზიის შემთხვევა საქართველოში

ქ. ნიკოლაიშვილი*, ც. ლომიძე*, ლ. მურვანიძე*, ლ. არაბული*, ქ. ასათიანი*

*ილიას სახელმწიფო უნივერსიტეტი, ზოოლოგიის ინსტიტუტი, თბილისი, საქართველო

(წარმოდგენილია აკადემიის წევრის თ. სადუნიშვილის მიერ)

კვლევის მიზანს წარმოადგენდა საქართველოს სამხრეთით, ჯავახეთის პლატოზე გავრცელებული ბრუცების ჰელმინთოლოგიური გამოკვლევა. ასპინძისა და ახალქალაქის მუნიციპალიტეტების ტერიტორიებიდან მოპოვებულ სამ ბრუცა - ***Nannospalax xanthodon***-ის შინაგანი ორგანოების გამოკვლევისას, წვრილ ნაწლავებში ნაპოვნი იქნა მხოლოდ ცესტოდები. დაინვაზიებული აღმოჩნდა სამივე მასპინძელი ცხოველი. ინვაზიის ინტენსივობა იყო 1-2 ცესტოდა ერთ ბრუცაში. ამ ჯგუფის ცხოველებისთვის ცესტოდოზის შემთხვევა პირველია საქართველოში. ბრუცა - ***N. xanthodon***-ში გამოვლენილი ცესტოდების მორფოლოგიური მახასიათებლების: სკოლექსი, კისერი, პროგლოტიდები, კვერცხები – მორფომეტრული შესწავლის შედეგად და ლიტერატურულ მონაცემებთან შედარებითი ანალიზის საფუძველზე დადგინდა, რომ ცესტოდები მიეკუთვნება **Cyclophyllidea (Braun,1900) – რიგს, Anoplocephalidae (Cholodkowsky, 1902) – ოჯახს და *Paranoplocephala* Lühe, 1910 გვარს.**

REFERENCES

1. Andreiyko O.F. (1963) K izucheniyu parazitofauny srednego slepisha *Spalax leucodon* Nordmann. *Parazity zhivotnykh i rastenii Moldavii*, 10-18 (in Russian).
2. Andreiyko O.F.(1987) Gelmintologicheskaiia kharakteristika slepishei (Rodentia: Spalacidae) fauny Sovetskogo Soyuza.V Zakavkazskaia konferentsia po parazitologii. Erevan, Tezisy dokladov, 71-72 (in Russian).
3. Fair J. M., Schmidt G.D., Wertheim G. (1990) New Species of Andrya and Paranoplocephala (Cestoidea: Anoplocephalidae) from Voles and Mole-Rats in Israel and Syria. *J. Parasitol.* **76**, (5): 641-644. American Society of Parasitologists.
4. Tinnin D.S., Ganzarig S., Gardner S.L. (2011) Helminths of small mammals (Erinaceomorpha, Soricimorpha, Chiroptera, Rodentia, and Lagomorpha) of Mongolia. Special publications Museum of Texas Tech University, 59.
5. Froeschke G., Matte S. (2014) Landscape characteristics influence helminth infestations in a peridomestic rodent-implications for possible zoonotic disease. *Parasit Vectors*, **7:** 393. DOI:10.1186 /1756-3305-7-393.
6. Vlasov E. A., Malysheva N.S., Krivopalov A.V. (2015) Helminth fauna of Myomorph rodents (Rodentia, Myomorpha) in the Central Chernozem State nature reserve. *Russian Journal of Parasitology*, **4:** 24-33. DOI: 10.737/16656.
7. Martinez- Salazar E.A., Flores-Rodriges V., Rosas-Valdez R., Colcon- Ordaz J. (2016) Helminth parasites of some rodents (Cticetidae, Heteromyidae, and Sciuridae), from Zacatacax, Mexico. *Revista Mexicana de Biodiversidad*. **87**, Issue 4: 1203-1211 https;//doi.org 10.1016/j.rmb.2016.10.009.
8. E-zenwa V.O. (2016) Helminth - microparasite co-infection in wildlife: lessons from ruminants, rodents and rabbits. *Parasite Immunolog*., Issue 9: 527-534.https://doi.org/10.1111/pim.12348
9. Archer E. K., Bennet N.C., Junker K., Faulkes C.C., Lutermann H.(2017) The distribution of gastrointestinal parasites in two population of common mole-rats (*Cryptomys hottentotus hottentotus*). *J. Parasitology*, **103**(6): 786-790. DOI: 10.1645/17-62.
10. Kirshenblat Y.D. (1938) Zakonomernosti dinamiki parazitofauny myshevidnykh gryzunov. 1-92, Leningrad (in Russian).
11. Kirshenblat Y.D. (1941) Novyi lentochnyi cherv iz zakavkazskikh polevok *Andrya* (s.str.) *montana*.sp.n. *Soobshenia Akademii Nauk Gruz.SSR*. **II,** 3: 273-276 (in Russian).
12. Kirshenblat Y.D. (1948) Materialy k gelmintofaune gryzunov Gruzii. *Trudy Zool. Instituta AN GSSR*, **VIII:** 317-339 Tbilisi (in Russian).
13. Kirshenblat Y.D. (1948a) New data about larval stages of tapeworms in rodents of Georgia. *Bull. Acad. Sci. Georgia*, **IX,** 4: 269-271(in Georgian).
14. Matsaberidze G.V. (1976) The helminths of the micromammalians of Georgia (fauna, systematics, morfologia,ecologia, zoogeography,medical-veterinary value). 235 p. Tbilisi (in Georgian).
15. Prokopić J. Matsaberidze G. (1972) Cestodes species new for the parasite fauna of micromammalians from Georgia.*Véstnik Československé společnosti Zool.* **36** (3): 214-222.
16. Kurashvili B. E., Matsaberidze G.V., Sadykhov I.A., Rodonaiia T. E. (1989) Parasiticheskie chervi melkikh mlekopitaiushikh fauny Zakavkazia. 197p. Tbilisi (in Russian).
17. Tamam O.A.S. (2014) Parasitic perifollicular dermatitis in the Egyptian lesser Blind Mole-Rat (*Spalax leucodon egyptiacus*). *Bangl. J.Vet. Med.* **12** (2): 197-201. ISSN: 1729-7893(Print), 2308-0922 (Online).
18. Nikolaishvili K., Lomidze Ts., Murvanidze L., Arabuli L., Asatiani K. (2019) First finding of cestodes in mole-rat *Nannospalax nehringi* (Satunin, 1898) in Southern Georgia. *Bio Eco 2019 – International Biodiversity and Ecology Sci. Sym.* 26-28 Sept. Istanbul. (Turkey), p.349. http://bio eco 2019. com.
19. Bukhnikashvili A. et al. (2017) Nehring's Blind Molerat// RED List IUCN https://www.iucnredlist.org/species/14327/113304138.
20. Rausch R.L. (1976) The genera *Paranoplocephala* Lűhe, 1910 and *Anoplocephaloides* Baer.1923. (Cestoda: Anoplocephalidae), with particular reference to species in rodents. *Annales de Parasitologie Humaine et Comparee* 51, 5: 513-562. https://doi.org/10.1051/parasita/1976515513
21. Ryzhikov K. M., Gvozdev E.V., Tokobaev M.M., Shaldybin L.S., Matsaberydze G.V., Merkusheva I.V., NadtochiyE.B., Khokhlova I.G., Sharpilo L.D. (1978) Opredelitel gelmintov gryzunov fauny SSSR. Tsestody i trematody. 232 M. (in Russian).
22. Gubányi A., Murai Ė. (2002) New Anoplocephalid parasites (Cestoda: Anoplocephalidae, Paranoplocephala) from the Fertö-Hanság National park.The fauna of the Fertö-Hanság National Park, 111-120. Hungarian Natural History Museum, Budapest.
23. Yanchukov A., Kandaurov A., Şahin K., Matur F. (2020) Karyotype analysis of *Nannospalax xantodon (*Spalacidae,Rodentia) at the Easternmost part of its distribution range. *Commun Fak. Sci. Univ. Ank. Series* C. **29 (**1): 61-70.
24. Wertheim G., Nevo E. (1971) Helminths of birds and mammals from Israel: III. Helminths from chromosomal forms of the mole-rat *Spalax ehrenbergi*. *Journal of Helminthology* **45:**161-169.

25. Haukisalmi V., Henttonen H. (2003) What is *Paranoplocephala macrocephala* (Douthitt, 1915) (Cestoda: Anoplocephalidae)? *Systematic Parasitology*, **54:** 53-69.
26. Haukisalmi V., Henttonen H. (2007) A taxonomic revision of the *Paranoplocephala primordialis* (Douthitt) complex (Cestoda: Anoplocephalidae) in voles and squirrels. *Zootaxa*, **1548** (1):51-68. DOI.10,11646/Zootaxa.1548.1,2.
27. Haukisalmi V., Hardman L. M., Hoberg E., Henttonen H. (2014) Phylogenetic relationships and taxonomic revision of *Paranoplocephala* Lűhe, 1910 sensu lato (Cestoda, Cyclophillidea, Anoplocephalidae). *Zootaxa* **387** (4): 371-415.DOI: 10. 11646/Zootaxa 3873, 4.3.

Received December, 2021