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SKELETON CHANGES IN THE ORIBATID MITES UNDER THE INFLUENCE OF THE HEAVY METAL IONS ACCUMULATION

ИЗМЕНЕНИЯ ПОКРОВОВ КЛЕЩЕЙ ОРИБАТИД ПОД ВЛИЯНИЕМ НАКОПЛЕНИЯ ИОНОВ ТЯЖЕЛЫХ МЕТАЛЛОВ

H.V.Dubinina, A.N.Alekseev
Е.В.Дубинина, А.Н.Алексеев

Zoological Institute, Russian Academy of Sciences, St.Petersburg, Russia 199034
Зоологический институт Российской академии наук, Санкт-Петербург, Россия 199034

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Ключевые слова: клещи-орибатиды, накопление ионов тяжелых металлов, аномалии, покровы, конечности, асимметрия.

ABSTRACT

Among oribatid mites collected in the polluted area in the vicinity of St.Petersburg, some anomalies of their skeleton were checked. It was supposed that such anomalies might be connected with the high level of the heavy metal ions in the environment. Experiments with *Oppiella nova* cultivated on the substratum soaked by $NiCl_2$ solutions confirm this supposition. Quantity of the different kinds of leg and notogaster abnormalities increase among progeny of poisoned parents. It is supposed that this phenomenon may have resulted from the accumulation of Ni ions in the exoskeleton of the mites.

РЕЗЮМЕ

Среди клещей орибатид, собранных в загрязненных зонах в окрестностях Санкт-Петербурга, были обнаружены некоторые аномалии их скелета. Высокое содержание ионов тяжелых металлов во внешней среде позволило предположить, что эти аномалии связаны с их влиянием. Эксперименты с клещами *Oppiella nova*, культивируемыми на субстрате, смоченном растворами $NiCl_2$, подтвердили это предположение. Число различного рода аномалий конечностей и покровов возрастало среди потомства отравленных родителей. Высказано предположение, что этот феномен может быть результатом накопления ионов никеля в покровах клещей.

INTRODUCTION

Anomalies in the bloodsucking ixodid ticks were described as early as a detailed description of the species itself appeared [Neumann, 1899]. Duplication of some organs [Warburton, Nuttall, 1909] and hynandromorphism [Joan, 1919] were described in the very beginning of the XXth century. In Russian literature, the most detailed classification of various anomalies of the exoskeleton and organs was produced by Academician E.N.Pavlovsky [1939] who was one of the first to underline that some abnormalities during tick metamorphosis can be connected with the influence of some exogenous factors. At that time nobody connected them with environment pollution. This factor is very important for the soil mites, especially for Oribatids, because they not only live in the soil which accumulates practically all polluting factors, but mostly have as their main food resource the fungi, well known accumulators of many pollutants, including heavy metal ions.

Investigation of the norm and pathology among oribatid mites presents serious difficulties mainly because the description and classification of this group of arthropods is very far from being completed. It is not finished even for the Leningrad Region where our investigation was conducted, despite the fact that for this area we have one of the most complete descriptions of oribatid fauna produced by L.G.Sitnikova [1962] who describes as many as 191 species. The situation with species description is complicated not only because of the totally new species emergence, but also due to the revision of the existing classification [Balogh, 1983].

It seems that anomalies among oribatid mites were studied by F.Grandjean only [1928 – 1974] who investigated the differences among mite clones from various parts of their distribution area. As for the anomalies in their exoskeleton structures, this author tried to connect them with some mutagenic effects of environment important for the metamorphosis of mite species studied, but did not relate these anomalies to any definite mutagenic active substrates.

It is known only that polluted sewage waters decreased the fauna and abundance of oribatid mites [Bhattacharya, Bhattacharya, 1983], which are very susceptible to SO_2 in the environment [Lebrun et al., 1977], and that under the influence of the heavy metal ion accumulation the variety and abundance of fungi [Nordgren et al., 1983], the feeding source of oribatid mites, are also changing. Oribatid mites are the single group of animals in the soil for which it is possible to digest, utilize and return into the food chains dead fungi and their spores. Therefore it is not strange that oribatid mites are able to accumulate a very high concentration of the heavy metal ions, for example $Pl(NO_3)$ and $CdCl_2$ [Ludwig et al., 1991], and to detoxicate them. For our purposes it was very important to recognize that Ludwig et al. supposed that at least one place of the metal ion accumulation is the mite's cuticle.

The purpose of our investigation is to demonstrate that the consequences of such accumulation are visible as some sort of changes in oribatid mites exoskeleton, which are passed on to their progeny.

MATERIALS AND METHODS

Oribatid mites were collected using standard technique of $10 dm^3$ soil samples in the different parts of the most polluted area of the Leningrad Region. Arthropods migrating from the warmed Tulgren funnel were collected either in 70% ethanol for the fixation and species determination, or in the water to collect living specimens. Some of soil samples were studied to determine the content of some heavy metal ions. Mites fixed in the Fora liquid were classified and checked for the existence of abnormalities, living ones were cultivated. Because clear abnormalities were detected only among the representatives of Oppioidea, specimens of one of them, *Oppiella nova*, were cultivated and tested for their ability to accumulate heavy metal ions and for some kind of abnormalities during metamorphosis and among their progeny.

Into the small glass cups, 2.5 cm in height and 3.5 cm in diameter, by 1/3 filled with a moist mixture of the plaster and activated charcoal 0.7-1.0 g potato slices were placed soaked in $NiCl_2$ solutions of varying concentration.The concentrations of $NiCl_2$ (calculated by the pure mass of Ni) were 1, 10 and 100 sanitary limit norm doses (LND) in the environment (we shall call them not LND, but 1, 10 and 100 PDK, as is usual in Russia).

The quantities of solution absorbed by the potato slices were determined by weighing. Into each cup on the potato slices 50 adults of *Oppiella nova* from the culture were placed by the thin squirrelfur brush. Larvae and nymphs were reared on the same medium, and emerging adults of F1 were checked and counted. Before the hatching of adults from the immobile tritonymphs, their parents were put into new cups with a fresh medium and fresh $NiCl_2$-soaked potato slices, not to mix two or more generations of the mites.

This manipulation was repeated thrice because of the parent mites longevity. The progeny was analyzed one by one ("periods" in the Tables 3-4) to check the differences in the nature and number of abnormalities as a function of the parent life duration and of the period of feeding on the poisoned potato slices. Results of the first series of experiments with 0, 1, 10, and 100 PDK are shown in the Table 2, other results in the Tables 3-4 (10 and 100 PDK).

Some part of the F1 adults were then placed in the 70% ethanol and investigated under the microscope to determine the quantity and nature of abnormalities. The development duration was also checked. Another part of the F1 adults and their progeny cultivated on the unpolluted substratum were also checked for the presence of abnormalities. Then the quantity of abnormalities among F2 adults were calculated and compared with that in F1 ones.

RESULTS

As seen from the Table 1, the quantity of heavy metal ions in all samples was many times greater than the limit level, especially for Ni and Pb ions. In all samples the detected level was at least ten times higher than it is permitted by the sanitarian norms. Given such a high level of pollution, it seems a bit strange that we met with abnormalities only four times among more than 5 thousand investigated specimens. A possible explanation of this rarity of detection consists in the difficulty of detecting abnormalities especially among the heavy "chitinized" species of Oribatida. Detection of abnormalities in the Oppioidea group is possibly the result of the better translucency of the cuticle. Fig.1 shows

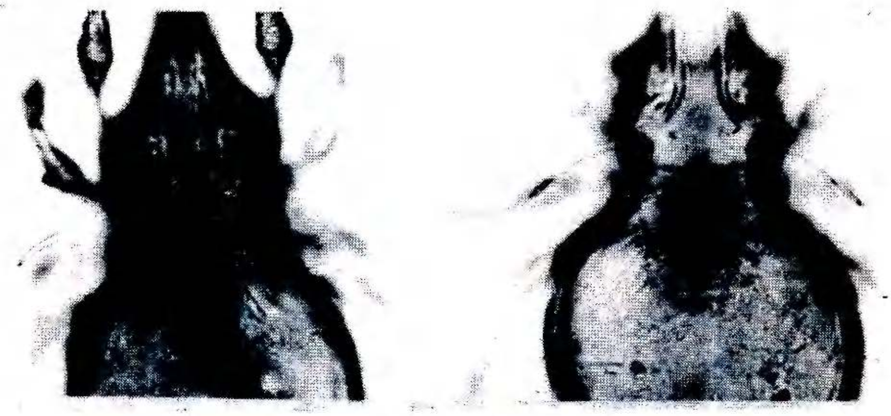

Fig.1. Normal *Autogneta* sp. (Oppioidea) mite (left) and mite with the anomalies of the notogaster and trichobotria (right). General view.
Рис.1. Нормальный (слева) клещ *Autogneta* sp. (Oppioidea) и клещ с аномалиями нотогастра и трихоботрии (справа). Общий вид.

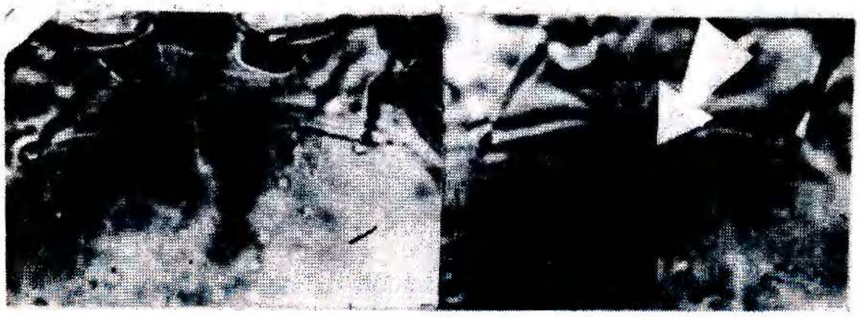

Fig.2. Enlarged view of the anterior notogaster edge of the normal (left) and anomalous (right) specimen.
Рис.2. Передний край нотогастра нормальной (слева) и аномальной (справа) особей.

(left) a normal mite *Autogneta* sp. and (right) a specimen with the displasia of the anterior border of the notogaster and a deformation of the left sensory organ (trichobotria). In Fig.2 and Fig.3, enlarged views of the notogaster edge and the left trichobotria are shown.

Some deformations of the exoskeleton were checked among *Oppiella nova* specimens cultivated on the potato slices. Deformations of the femur

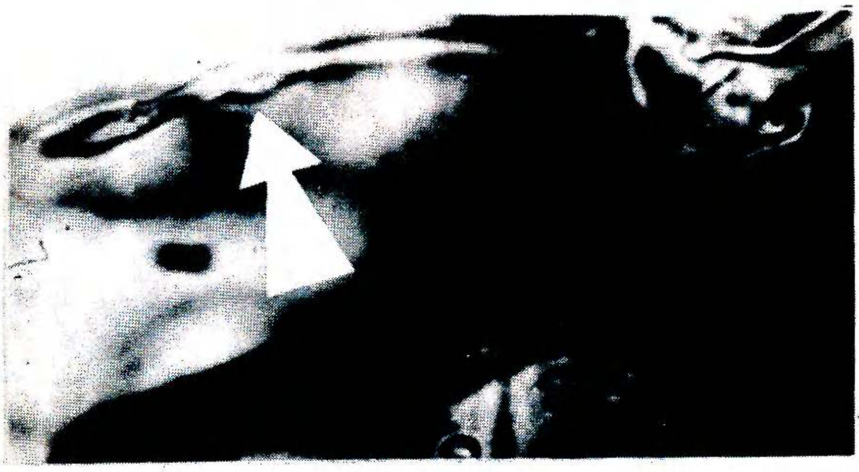

Fig.3. Anomaly (thickening) of the left sensory organ (trichobotria) of *Autogneta* sp.
Рис.3. Аномалия (утолщение) левого сенсорного органа — трихоботрии *Autogneta* sp.

which were sometimes met in the parent culture (Table 2 and 2, 3 in Fig.4) quickly accumulated under the influence of the $NiCl_2$ in the food. It is of interest that tenfold increase in the anomalies of legs as a result of tenfold increase in the $NiCl_2$ concentration (compare control and 10 PDK results: 1.6 and 14.5 in Table 2) did not repeat on the second tenfold rise of $NiCl_2$ concentration. Percentage of anomalies is equal to only 15.2. At the same time the rate of two and three leg deformations present simultaneously is much higher than with 10 PDK. It seems that specimens with multiple anomalies simply did not develop to the adult phase, dying in the preimaginal phases. $NiCl_2$ has only a slight inhibitory effect on the speed of the mite embryonic development (3-5 days), and such inhibition was possible to check only in the first period of investigation. Then any difference disappeared, possibly as a result of the downfall of the susceptible specimens of parent mites (50-64% death rate vs. 44% in the control). Maybe because of the same reason it is possible to observe a small increase in fertility of nickel chlorid treated mites. But the increased quantity of exoskeleton anomalies (Tables 2-4) means that $NiCl_2$ is a very important pollutant of the environment for such an important link of the food and energy chains as oribatid mites.

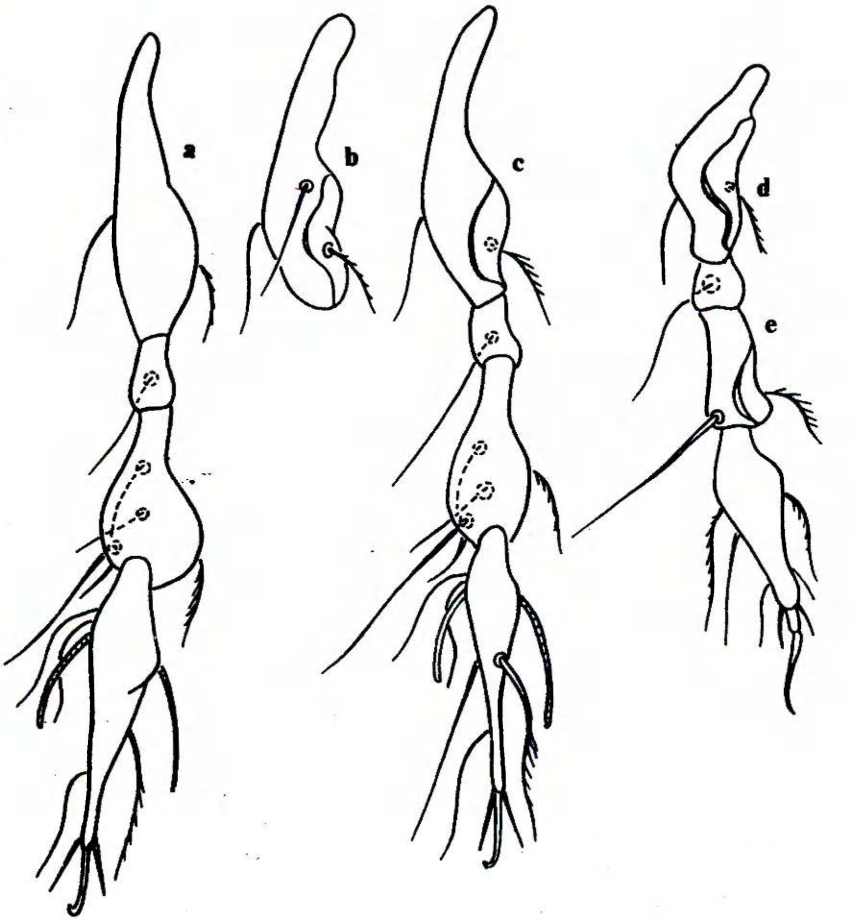

Fig.4. Anomalies on the legs of *Oppiella nova*.
a — normal leg; b — femur of the first leg with anomaly, back view; c — same, ventral view; d — anomalies on the femur of the second pair of the legs; e — same, on the tibia.

Рис.4. Аномалии конечностей *Oppiella nova*.
a — нормальная конечность; b — бедро первой пары конечностей с аномалией, вид сзади; c — то же, вид спереди; d — аномалия бедра второй пары конечностей; e — то же на голени.

Table 1. Soil samples containing oribatid mites

Sample characteristic	Heavy metal ion quantity (x-ray-fluorescent analyzes and sanitarian limits (in the bottom)				Samples pH	Arthropods							
	Pb 7E-4	Zn 1.6E-3	Ni 2E-4	Fe 3.8E-1		number in cub. dm	oribatid quantity						
							families	species	specimens			specimens with abnormalities	
									total	Oppioidea			
										abs.	%	abs.	%
Aspen, spruce, young birch, fir, fallen leaves	10E-3	4E-3	<3E-3	2.3	3.2	2870	22	35	1878	382	20.3	3	0.15
Water-logged rarer grove, aspen, sphagnum, moss, bilberry	17E-3	1.5E-7	3E-3	6.7	4.5	3799	9	24	3367	616	18.3	1	0.03
Sidepart of road, weed	1.3E-3	5E-3	<3E-3	1.5	6.2	767	5	5	225	91	40.4	—*	—
Sum of specimens investigated for abnormalities									5470	1089	19.9	4	0.07

E — the exponent; * — pathology among larvae of *Dasyhelea* sp. (Ceratogonidae).

Changes in the chitinous covers of mite legs	NiCl2 concentration (in PDK)							
	0		1		10		100	
	abs.	%	abs.	%	abs.	%	abs.	%
On one femur:								
— of one leg	2	66.6	4	80.0	9	24.3	9	34.6
— of two legs	1	33.3	1	20.0	—	—	1	3.8
On the two femora:								
— of one leg	—	—	—	—	24	64.9	2	7.7
— of two legs	—	—	—	—	—	—	5	19.2
— of three legs	—	—	—	—	—	—	2	7.7
On the femora and tibiae:								
— of one leg	—	—	—	—	1	2.7	—	—
— on femora of two legs and tibia of one	—	—	—	—	1	2.7	2	7.7
— of two legs	—	—	—	—	1	2.7	1	3.8
— on femora of three legs and tibia of one	—	—	—	—	1	2.7	2	7.7
— on femora of three legs and tibiae of two	—	—	—	—	—	—	2	7.7
Total number of the changes	3/190	1.6	5/179	2.8	37/255	14.5	26/171	15.2
The body side on which at least single changes of the leg cover were checked,%	only on the right side						right 80.8 left 19.2	

← Table 2.
Nature and frequency of the *Oppiella nova* exoskeleton changes under the influence of $NiCl_2$ ions

In the second series of the experiment (Table 3) we were taking into account not only leg anomalies, but also changes in the notogaster. The latter are in the direct correlation not only with $NiCl_2$ concentration (37 and 46% at 10 and 100 PDK, respectively), but depends also on the duration of the period of investigation. Heavy metal ion accumulation during parent mites individual life leads to the increased notogaster abnormality frequency. According to the quantity of the progeny in the third period, such accumulation is not indifferent to the parents: the quantity of the progeny in the third period (100 $NiCl_2$ PDK) decreased not only in comparison with the first and second ones in the same experiment, but also in comparison with the fertility of mites bred on the ten times lower concentration of $NiCl_2$. It means that sum of leg and notogaster anomalies is a very good compound index of the manifest toxic and possible latent mutagenic effect of the heavy metal ions.

From Table 4 it is clear that the highest rate of the multianomalous leg skeleton among all cases of leg anomalies decreased from the first to the third period. It is possible to imagine that their increase is accompanied by other physiological changes which are incompatible with the life: 24 anomalies (the highest observed level) of 32 possible ones on the leg links – were observed only once among 674 investigated specimens (0.14%).

Among F2 of the control mites which were bred on the clean (not natural) substratum, no leg anomalies were observed and abnormalities of the notogaster were observed only in 1 case among 16 inspected specimens (6.2%). On the opposite, the F2 progeny whose parents and grandparents were bred on the substratum with 100 $NiCl_2$ PDK had notogaster and compoud abnormalities in 50% of cases, and multiple and bilateral anomalies were checked as often as in 33% of cases. It seems that the main cause of mite pathology is the result of the toxic action of Ni ions. But there is another interesting aspect of the problem: the asymmetry of the exoskeleton lesions. From Table 2 it can be seen that in the control part of the mite population and in the experiments with low $NiCl_2$ concentration leg lesions were observed only on one side of the

Table 3.
Abnormalities among the progeny of mite parents fed on the $NiCl_2$ polluted substrate

Periods of oviposition	Anomalies among F1						No mites
	legs		not-ogaster		legs+notogaster		
	abs.	%	abs.	%	abs.	%	
10 PDK NiCl2 in F1 substrate							
I	5	5.9	27	31.8	32	37.6	85
II	11	21.6	9	17.6	20	39.2	51
III	3	2.97	52	51.4	55	54.4	101
All	19	8.01	88	37.1	107	45.1	237
100 PDK NiCl2 in F1 substrate							
I	14	9.3	58	38.6	72	48.0	150
II	13	5.7	111	48.9	124	54.6	227
III	7	11.6	32	53.3	39	65.0	60
All	34	7.8	201	46.0	235	53.7	437

body. Increased $NiCl_2$ concentration produced bilateral leg anomalies (less than 20%, Table 2). Continuation of the heavy metal ion action increased the quantity of bilateral anomalies to 33%.

CONCLUSION

Increased mite leg abnormalities can be explained either by the genetically based changes [Palmer, Strobeck, 1992] or by the complicated result of the Ni accumulation in the exoskeleton structures of the mite progeny. It would be of interest to study how long after the cessation of the nickel chlorid press such abnormalities would continue to appear. We demonstrated it only in F2, but the supposition that some quantity of the leg and notogaster abnormalities can exist much longer seems to us quite plausible.

The possible explanation of the checked by us phenomenon consist in the supposition that there exists the process of the superseding of the normally used for the cuticle hardening calcium oxalate ingested from the normal fungi [Norton, Behan-Pelletier, 1991] by the nickel spoiled salts from the $NiCl_2$ poisoned food. The quantity of calcium in the different

Table 4.
Comparison of the case frequency of the single- and multianomalous legs as function of the $NiCl_2$ concentration

Anomalies of legs	NiCl2 concentration, periods							
	0 (control)		10 PDK			100 PDK		
	I	II	I	II	III	I	II	III
On one leg	2	—	1	1.3	1	1	1	1.3
On two legs	2	—	3	3	2	2	2	3
Many (from 3 to 8)	7	4	6	6.86	0	7	5.2	12.3
Maximum no. of anomalies				8		18		24*
The rate of multianomaly: abs.	1/3	2/2	6/9	7/11	0/3	10/13	5/8	3/7
%	33.3	(100)	66.6	63.6	0	76.9	62.5	42.8

*on all parts of all legs, it means 24 from the 32 possible ones.

groups of Oribatida fluctuates from 0.11 mg/kg in *Plathynothrus* sp. to 71.3 – in *Liacarus* sp. [according Pokarzhevskiy, 1985], but its including in the skeleton hardening seems obvious as well as its substitution by the other heavy metal ions.

Independently of the background of investigated phenomena, it is clear that accumulation of the heavy metal ions (in our case, $NiCl_2$) is the cause of some serious biochemical, physiological and morphological changes in mites and probably in ticks [Alekseev, Dubinina, 1993] which may have some additional and possibly serious consequences not only for the food and energy chains, but also for some human disease agents transmission.

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