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CONTENTS

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Biochemistry

INHIBITORY EFFECT OF PROANTHOCYANIDIN PREPARATION FROM TEA LEAVES ON TEA LEAF PHENOLOXIDASE ACTIVITY

MCHEDLISHVILI N., OMIADZE N., AKHVLEDIANI K., ABUTIDZE M., GULUA L., PRUIDZE N.

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(Received September 5, 2011)

Abstract

The effect of the preparation of proanthocyanidins isolated from leaves of green tea (*Camellia sinensis* L.) on the activity of tea leaf phenoloxidase has been studied. The preparation of proanthocyanidins from tea leaves has been shown to be effective natural inhibitor of tea leaf phenoloxidase. At about 0.025mg/ml concentration of the inhibitor 50% inhibition was observed. The addition of the natural inhibitor caused deviation of the kinetics of the enzyme from classical kinetics. The natural inhibitor decreased V_{max} of phenolxidase 2.5–fold. The enzyme showed positive cooperativity. The Hill coefficient n_H was found to be 0.63. The inhibition of tea leaf phenoloxidase by the natural inhibitor from tea leaves was non-competitive.

Key words: tea leaves, phenoloxidase, natural inhibitor, kinetics

Introduction

Phenoloxidase catalyzes two different basic reactions: monophenol hydroxylation into *o*-diphenols (hydroxylase activity) and dehydrogenation of *o*-dioxysubstituted polyphenols (catechol oxidase activity) [Rodriguez-Lopez et al, 2000, Fenoll et al, 2000]. In both reactions molecular oxygen is used as a co-substrate. The currently accepted enzyme nomenclature classifies the hydroxylating phenoloxidase as monophenol monooxygenase (EC.1.14.18.1) and the *o*-diphenols oxidizing phenoloxidase as catechol oxidase (CO; EC 1.10. 3.1) [Rompel et al, 1999].

Although a large number of phenoloxidase inhibitors have been described in literature [Moon et al, 1999; Kubo et al., 1997; Lee G. & Lee C., 1997], the search for new natural products with such activities still continues [Kubo et al, 2000]. We previously reported about the isolation of crude preparation of proanthocyanidins from leaves of green tea (*Camellia sinensis* L.) and its inhibitory effects on apple phenoloxidase and peroxidase [Omiadze et al., 2004].

The aim of the present work was to examine the inhibitory effects of the preparation of proanthocianidins isolated from green tea leaves on the activity of tea leaf phenoloxidase.

Materials and Methods

Fresh tea (*Camellia sinensis*) leaves were used in the experiments. The tea leaves were field-collected in West Georgia.

Isolation of phenoloxidase natural inhibitors from tea leaves was carried out as described in [Omiadze et al., 2004].

Isolation of phenoloxidase crude preparation from tea leaves was carried out as described in [Mchedlisvili et al., 2005].

Protein content was determined by the method using Amino Black reagent [Plum et al., 1955].

Phenoloxidase activity was determined spectrophotometrically according to the increase in absorbance at 420 nm and expressed in arbitrary units [Lanzarini et al., 1972]. The incubation time was 5 min and one unit was defined as the amount of the enzyme sufficient to change the absorption spectrum by 0.05 within 1 min. The specific activity was expressed in units per mg protein.

Results and Discussion

The effect of different concentrations (0.01-0.2 mg/ml) of crude preparation of proanthcyanidins from tea leaves on tea leaf phenoloxidase (0.001-0.04 mg/ml) activity was studied. The tested preparation was found to be a potent inhibitor of the phenoloxidase activity without pre-incubation and when added directly to the reaction mixture before addition of the enzyme. Phenoloxidase activity was gradually decreased by increasing the concentration of the natural inhibitor from tea leaves; at about 0.025mg/ml concentration of the inhibitor 50% inhibition was observed (Fig.1)

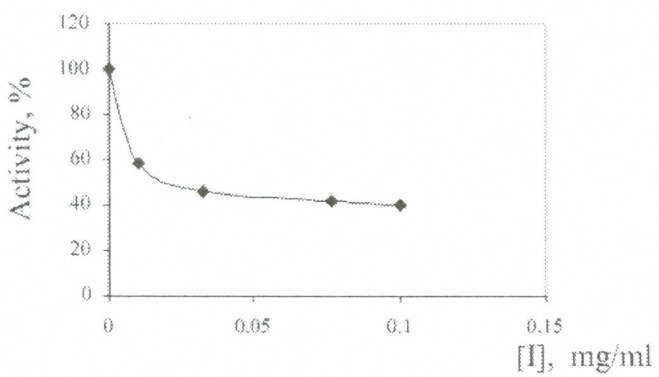

Fig. 1. Effect of different concentration of natural inhibitor from tea leaves on tea leaf phenoloxidase activity

As shown in Fig. 2, the inhibition of tea leaf phenoloxidase by the natural inhibitor from tea leaves was pH-dependent.

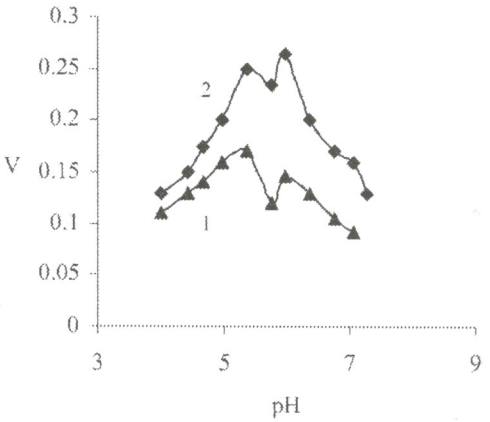

Fig. 2. Effect of pH on tea leaf phenoloxidase activity with (1) and without (2) natural inhibitor from tea leaves (V - specific activity, ΔE/mg protein/min).

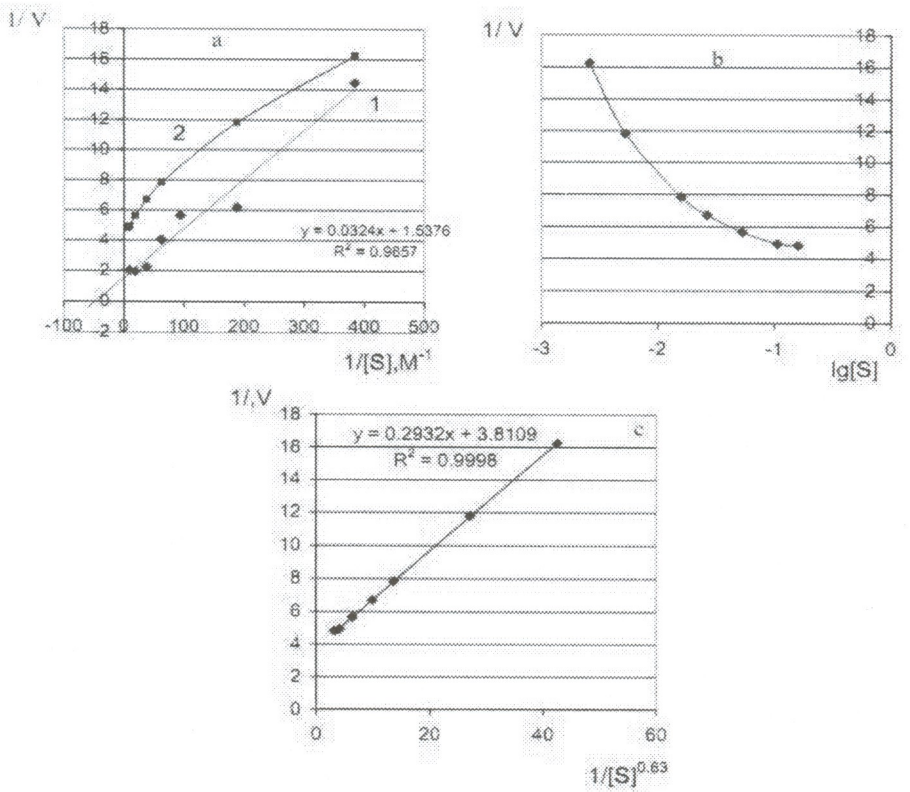

Fig.3. Effect of substrate (catechol) concentration on tea leaf phenoloxidase activity (a) without (1) and with (2) natural inhibitor from tea leaf. Determination of $V_{max(i)}$ and K_{05} (b, c)

Phenoloxidase reaction without natural inhibitor was described by the classical kinetics (Fig. 3a). V_{max} and K_m were calculated from Lineweaver–Burk plots. V_{max} = 0.65 ΔE/mg

protein/min and K_m =20 mM. In the presence of the natural inhibitor from tea leaves the hyperbolic curve of the dependence of the enzymatic reaction initial rate on substrate concentrations changed into a sigmoid one. The enzyme showed positive cooperativity The Hill coefficient value n_H was found to be 0.63. By expressing the experimental data in $\frac{1}{[S]^{n_H}} - \frac{1}{V}$ coordinates, $V_{max(i)}$ and $K_{0.5}$ were calculated (Fig. 3c). $V_{max(i)}$ was found to be 0.26 ΔE/mg protein/min and $K_{0.5}$ was equal to 20mM.

By the action of the natural inhibitor from tea leaves the maximal rate of the phenoloxidase reaction decreased 2.5 fold, but the affinity towards substrate did not change. This fact indicates that inhibition of tea leaf phenoloxidase by the natural inhibitor from tea leaves was non-competitive.

Acknowledgements: This work was carried out with the financial assistance of Science and Technology Center in Ukraine within the framework of the project No: 4894

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ჩაის ფოთლიდან გამოყოფილი პროანტოციანიდინების პრეპარატის მაინჰიბირებელი გავლენა ჩაის ფოთლის ფენოლოქსიდაზას აქტივობაზე

მჭედლიშვილი ნ., ომიაძე ნ., ახვლედიანი ქ., აბუთიძე მ., გულუა ლ., ფრუიძე ნ.

საქართველოს აგრარული უნივერსიტეტის ს. დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი, თბილისი

(მიღებულია 05.09.2011)

რეზიუმე

გამოკვლეულია მწვანე ჩაის (***Camellia sinensis* L.**) ფოთლებიდან გამოყოფილი პროანტოციანიდინების პრეპარატის გავლენა ჩაის ფოთლის ფენოლოქსოდაზას აქტივობაზე. ნაჩვენებია, რომ აღნიშნული პრეპარატი წარმოადგენს ჩაის ფოთლის ფენოლოქსიდაზას ეფექტურ ბუნებრივ ინჰიბიტორს. მისი დამატება იწვევს ჩაის ფოთლის ფენოლოქსიდაზას მოქმედების კინეტიკის კლასიკურიდან გადახრას. ბუნებრივი ინჰიბიტორი ამცირებს ფენოლოქსიდაზას $V_{მაქ}$-ს 2.5 ჯერ. აღნიშნული ფერმენტი ამჟღავნებს დადებით კოოპერატიულობას. ჰილის კოეფიციენტი 0.63-ის ტოლია. დადგენილია, რომ ჩაის ფოთლის ფენოლოქსიდაზას ინჰიბირება ჩაის ფოთლიდან მიღებული ბუნებრივი ინჰიბიტორით არის არაკონკურენტული.

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Biophysics

STUDY OF PHYSICAL PROPERTIES OF SMITIN ISOLATED FROM SMOOTH MUSCLE AND COMPARATIVE ANALYSIS WITH THE SAME PROPERTIES OF TITIN

KUPATADZE R., KURIDZE K., DEVDARIANI M., GACHECHILADZE N., SULAMANIDZE L., IORAMASHVILI I., GOGORISHVILI J., ZAALISHVILI M.

Life Science Research Center

(Received October 24, 2011)

Abstract

The aim of our work was the obtaining and purification of native form of smitin (c-titin) from smooth muscle. Its physical parameters - molecular weight, characteristic viscosity and sedimentation coefficient, were determined. The comparative analysis with corresponding parameters of titin, received from chicken striated muscles was carried out.

Key-words: electrophoresis, sedimentation, viscozimetry, Smitin.

Introduction

Contraction is a complex biological process and it is realized through a highly organized apparatus, functioning of which depends on major (myosin, actin, tropomyosin), as well as on minor (troponin, actinins) and giant (titin, smitin) proteins.

It was established [Hoyle G., 1965] that in sarcomere besides thin and thick protofibrils there are third type of protofibrils of 25 Å diameter. Earlier fibriline, the main protein of protofibril, was found [Guba F., 1967], but unfortunately, this evidence has fallen into oblivion.

The protein isolated by Maruyama [Maruyama K., 1977] from the striated muscle was called as connectin. He has determined the molecular mass of this protein, but other characteristics have remained to be explored.

In our laboratory it was established that the molecule of connectin (by other authors this protein is named as titin) contains a collagen type structure (left helix) that determines firmness of myofibril [Kobakhidze G., 1984].

10% of the smooth muscle mass constitutes the giant protein titin. Titin in native form was obtained independently in three countries (England, USA, Japan) by [Trinick J.1984;Wang K. 1982; Maruyama K. 1981].

Titin consists of 27000 amino acids and its molecular mass is 3 million Dalton. Titin possesses immunoglobulin-like repetitive sites composed by 100 amino acids, and a unique section rich in proline, glutamine, valine and lysine that is called as PEVK. PEVK involves 1000–1200 amino acids. Immunoglobulin-like sites and PEVK function as the first and second order molecular springs.

Titin is stretched from Z-disk to M-band of the sarcomere and interacts with α-actinin and myosin. The titin threads of the striated muscle play a particular role in the organization of

sarcomere structure during myofibrinogenesis [Fulton A.,1991]. In the native striated muscle its elastic property maintains the central location for the thick threads in the sarcomere and resists passively the sarcomere's extension [Labeit S.,1992].

The structure and function of the striated muscle titin have been well studied.

PEVK interacts with actin, it slows down the movement of actin thread toward myosin. The skeleton muscle PEVK has less effect on actin movement than the cardiac muscle PEVK. The influence of PEVK on the cardiac muscle depends on Ca^{2+} concentration, whereas in case of skeletal muscle this dependence is not noticeable. In myofibrils actin-PEVK slight interaction forms a force which hinders the filament slide. Thus, PEVK is involved not only in stretch, but also participates in contraction [Wolfgang A. Linke, 2002].

It was demonstrated in 2002 that the smooth muscles contain a novel protein of 2 megadalton molecular mass which was called smitin much resembling the striated muscle protein titin by its molecular morphology and location within the contraction apparatus.

Smitin likewise titin represents a long fibrous molecule with a globular head at one end and molecular mass approximately 2000 kD. Antibody studies have revealed that smitin and titin do not represent one gene coded isoforms [Kim K., 2002].

Today there is insufficient information concerning the physical-chemical properties of smitin, it is only known that it interacts with myosin and alpha-actinin.

Smitin interacts with alpha-actinin at R_2 and R_3 spectrin-like repetitions and at EF-hand located at the end [Chi R. 2005].

During interaction with myosin smitin unexpectedly revealed diversity having formed different structural units in natural conditions. Smitin in vitro binds with myosin filaments, while in vivo it forms irregular groups and contains many "side polar" myosin threads. At low ionic strength it forms a special structure containing myosin bipolar threads. By immunoreaction and sedimentation it has been demonstrated that smitin and the smooth muscle myosin are associated with "side polar" and bipolar structures. It was shown that smitin plays a central part in the organization of myosin threads [Kim K. 2002].

The issue is extremely topical considering that smitin, likewise titin, as been noted above, has similar molecular morphology and location within the contraction apparatus, but its role in the smooth muscle tonic contraction is unexplained (there, in contrast to the striated muscle, the sarcomere is not distinctly formed and the contraction character is different).

The purpose of our work was the obtaining of native form of smitin (c-titin) from smooth muscle (stomach), and also the study of its molecular mass, sedimentation and viscosimetry. Since smitin has the similar molecular morphology and location within the contraction apparatus as titin, the comparative analysis with corresponding parameters of titin received from chicken striated muscles has been carried out.

Materials and Methods

Smitin was obtained from the chicken's smooth muscle, particularly from the stomach [Pan.K 1994]. We obtain the myofibrils from chicken stomach [Wang K. 1982]. Homogenized myofibrils (100g) processed with blender (10S) in A-buffer (2mM $MgCl_2$, 1mM EGTA, 0.5mM DDT, 2mM PMSF, 10mM imidazole, 10μM tripsin's inhibitor, 50mM KCl pH 7.0). Then the suspension of myofibrils was washed three times in A-buffer by centrifugation (5000g,10min, 4°C) and resuspended in extraction buffer B (2mM $MgCl_2$, 1mM EDTA, 0.6M KCl, 4mM ATP, 0.5mM DTT, 0.2mM PMSF, 10μg/ml tripsin's inhibitor, 10mM imidazole, pH 7.0) The extracted myofibrils were pelleted by centrifugation (15000g, 30 min., 4°C) and the supernatant was loaded onto a Toyopearl-65 (1.5 cm x 90 cm) column. The Toyopearl-65 column was equilibrated with

buffer (0.2M KCl, 10mM imidazole, 1mM EGTA, 0.5mM EDTA, 0.2 mM DTT, pH 7.5). Each fraction consists of 3 by 3 ml. The first fraction contained native smitin (c- titin) only, the second fraction contained smitin (c-titin) and myosin. Each fraction was studied by the electrophoresis in SDS-polyacrylamid gel (3-15 %). The protein concentrating was carried out on polyethylene glycol (M 4000-8000).

Electrophoresis in polyacrylamide gel (Laemmly). To determine homogeneity and molecular masses of protein fraction we used electrophoresis with the presence of Na dodecylsulphate in polyacrylamide gel [Laemmly, 1970], 3% acrylamide gel was used. Electrophoresis was accomplished in LKB2010 "Macrophor Sequencing System". Gels' scanning was realized on LKB-2202 densitometer (Sweden). Molecular mass was determined with the following standards: alpha-actinin – 100 kD, myosin heavy chain – 200 kD, nebulin – 800 kD, titin – 3000 kD.

Viscozimetry. Measurement of protein viscosity was made by means of Ostvald cappilar viscosimeter. Water flow time was 187min, 20°C. The volume of protein was 5 ml. The protein was in buffer (0.1 KCl, 0.05 M Tris/HCl, 1 mM βSH, pH7.5, T=20°C) during all experiments. The concentration of the protein chaneges from 0.9mg/ml to 0.3 mg/ml. Characteristic viscosity was calculated by the formula:

$\rho_{Charac}=(\rho/\rho_0-1)/C$ $C_{lim}\rightarrow 0$.

Sedimentation. Sedimentation analysis was carried out by the analytical centrifuge MOM-3180 with photosystem. The rotation speed was 31500 rot/min. (t=20°C), the protein was in buffer 0.1M KCl, 0.05M Tris/HCl, 1mM EDTA, 1mM βSH, pH=7.5, T=20°C. The concentration of protein was chaneged from 0.84 mg/ml to 0.5 mg/ml. The sedimentation coefficient was calculated by the formula:

$S=(1/\omega^2)*d(\ln r)/dt$ but for $S_{20,w}$ it was required to construct the curve S=f(C), $C_{lim}\rightarrow 0$.

Results and Discussion

In the first part of our studies we tried to show weather the different tissues (stomach, uterus, gullet) contain the protein smitin (c-titin) (Fig.1). The result has been checked up by the electrophoresis on the SDS polyacrylamide gradient gel 3-15% (Leammly). As we can see from electrophorogramme, in three cases the myofibrils contained the protein smitin. The electrophorogramme of myofibrils isolated from striated and smooth muscles are presented on Fig.2, from what follows that despite the similarity of morphology and location within contractile apparatus smitin and titin have various molecular weights (M_{Titin}-3000kD, M_{Smitin}- 2000kD).

By the method of sedimentation analysis it was shown that speed of sedimentation of smitin (c-titin) is less then that of titin (smitin $S_{20,\omega}$ =9.3S; titin $S_{20,\omega}$ =13,8S) which is also due to the higher molecular mass of titin (Fig.3).

The analysis of viscosimetrical data shows that the characteristic viscosity for titin and smitin is not high (smitin ρ_{Charac}=0.21; titin ρ_{Charac}=1.32) (Fig.4). The apparent low viscosity obtained during the experiment, is typical for globular proteins but we believe that this fact is explained by the elastic properties of the investigated proteins.

The elasticity factor isn't considered in the given article. The comparative study of elastic properties of these proteins we will publish in the near future.

Our work is the first step for explanation of a role of those proteins in cell cytoskeleton formation and their participation in the process of muscle contraction.

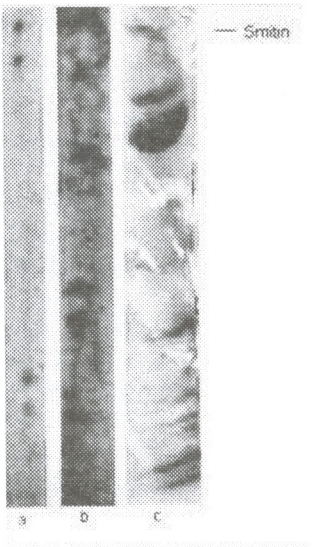

Fig.1. The electrophorogramme of myofibrils isolated from different tissues: a - stomach; b – uterus; c - gullet.

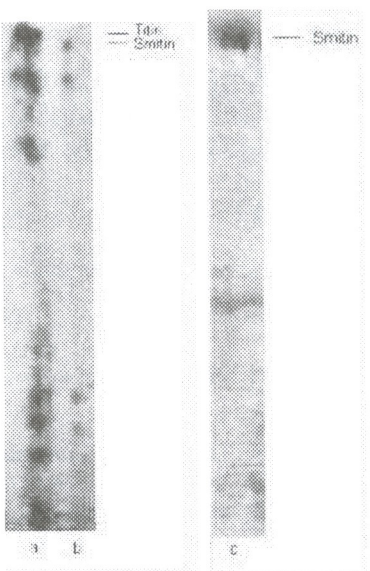

Fig.2. The myofibrils isolated from striated and smooth muscles: a - myofibrils isolated from striated muscles; b - myofibrils isolated from smooth muscles; c - the electrophorogramme of native smitin

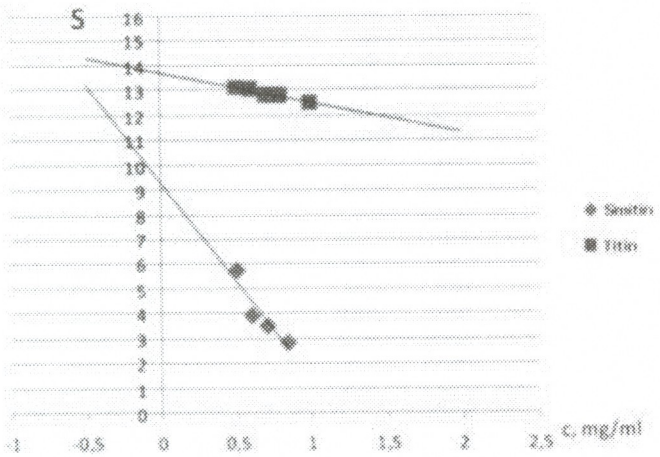

Fig. 3. The dependence of the sedimentation rate of smitin and titin on concentration.

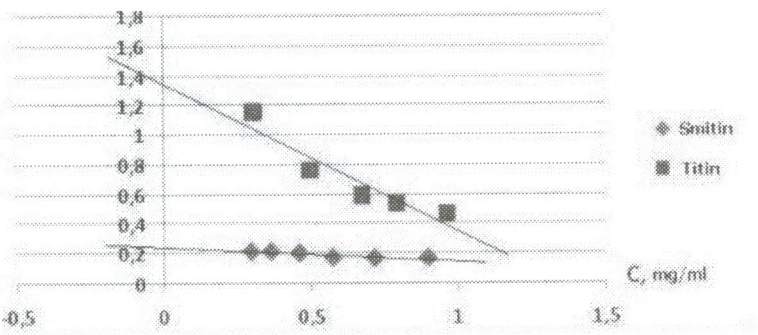

Fig. 4. The dependence of characteristic viscosity of smitin and titin on concentration.

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ქათმის გლუვი კუნთიდან გამოყოფილი ცილა სმიტინის ფიზიკური თვისებების შესწავლა და მათი შედარება ტიტინის ფიზიკურ თვისებებთან.

კუპატაძე რ., ქურიძე კ., დევდარიანი მ., გაჩეჩილაძე ნ., სულამანიძე ლ., იორამაშვილი ი., გოგორიშვილი ჯ., ზაალიშვილი მ.

სიცოცხლის შემსწავლელ მეცნიერებათა ცენტრი

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რეზიუმე

ჩვენს მიერ ქათმის გლუვი კუნთიდან ნატიური სახით გამოყოფილი იქნა ცილა სმიტინი (C–ტიტინი). დადგენილი იქნა მისი ფიზიკური პარამეტრები (მოლეკულური მასა, სედიმენტაციის კოეფიციენტი, მახასიათებელი სიბლანტე) და იგი შედარებული იქნა ქათმის განივზოლიანი კუნთიდან გამოყოფილი ცილა ტიტინის შესაბამის პარამეტრებთან.

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STUDY OF TOPINAMBUR (*HELIANTHUS TUBEROSUS L.*) FOR PRODUCING A FUNCTIONAL FOOD ADDITIVE

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Abstract

The topinambur plant introduced to Georgia was investigated to produce a functional food additive. Tubers of topinambur was shown to contain 92.0% extractive substances including 17.0 % inulin. The leaves of this plant was found to contain quite large amount of phenolic compounds (12.0%) and pectic substances (9.0%). Both tubers and leaves of topinambur contained sugars 7.0 and 13.0 % respectively. The regimes of extraction of inulin from topinambur tubers and phenolic compounds from topinambur leaves were determined. It was shown that topinambur tubers and leaves gathered in different regions of Georgia may be successfully used as raw materials for producing a functional biologically active food additive.

Key words: topinambur, inulin, phenolic compounds, functional food

Introduction

Increase of chronic diseases in the last decade is considered to be mainly connected with imbalanced nourishment. According to the recent data, cells need about 600 food components for normal functioning. Therefore, biologically active additives are being used in human diet more and more widely. Food additives with multifunctional properties are especially of great interest. For the content of physiologically active food ingredients, they will decrease the risk of development of different kinds of diseases or promote the health recovery process [Tikhomirova, 2009].

In this point of view the study of inulin-containing plants is very important. Inulin has been found to be desirable as a food or a food additive. It may offer a great number of health benefits, it has unique medicinal properties to cure different kinds of diseases. [Worawuthiyanan, 2007; Ohta et al., 2002].

The objective of this work was to investigate tubers and leaves of topinambur (*Helianthus tuberosus L.*) introduced to Georgia, as plant raw materials in order to obtain dry extracts of multifunctional biologically active food additive.

Materials and Methods

The tubers and leaves of topinambur introduced to Georgia were used as plant raw materials. The tubers were washed, cut into very small pieces, dried at 95-100°C and powdered. The leaves were fixed by water steam for 3-5 min, then dried and powdered.

Extractive substances, phenolic compounds, pectic substances and proteins were determined by standard biochemical methods [Jinjolia et al, 1983].

Total carbohydrates, soluble sugars and inulin were determined spectrophotometrically according to Beliakov&Popov [Beliakov & Popov, 1998].

To study the process of inulin extraction, the topinambur tubers were preliminary cut very small. The ratio of raw materials and extragent was equal to 1:100.

During the investigation of moving extractive substances and phenolic compounds to the extract, the extraction process was lasted 5 min, at 65-85°C. The ratio of raw material and water was - 1:5.

Results and Discussion

Tubers of topinambur was shown to contain high amount of extractive substances (92.0%) among them inulin was abundant (17.0%). In the leaves of topinambur the content of extractive substances was about 50.0%, but the content of inulin was slight (Table 1). The leaves of this plant was found to contain high amount of phenolic compounds (12.0%) and pectic substances (9.0%). The content of phenolic compounds in the tubers was found to be rather low (0.5%), while in the leaves it was more than 12.0%. Both tubers and leaves of topinambur contained sugars (7.0 and 13.0 % respectively).

Antioxidant activity of topinabur leaves was three times higher than that of topinambur tubers. This fact was certainly caused by the high content of polyphenolic compounds in the leaves of this plant.

Table1. Chemical composition and antioxidant activity of tubers and leaves of topinambur (% per dry matter).

№	Sample	Extractive substances	Phenolic compounds	Soluble pectic substances	Soluble sugars	Iinulin	Antioxidant activity [Fe^{+2}] $mM \times L^{-1}$
1	Tubers	92	0.5	21.0	7.0	17	0.3
2	Leaves	50	11.0	8.3	1.3	-	0.9

Accumulation of inulin in tubers of topinambur gathered in spring and autumn was also studied (Table 2). As it can be seen from Table 2, according to the seasonal accumulation of inulin there was no difference between the samples of topinambur tubers gathered in different regions.

Table 2. Seasonal accumulation of inulin in the tubers of topinambur grown in different regions of Georgia, % per dry matter

Region \ Season	Spring	Autumn
Marneuli	15. 2	16.1
Khulo	15.9	17.3

The effect of different factors on the extraction of inulin from topinambur tubers was studied. The results are given in Table 3.

It has been shown that maximum output of inulin from topinambur tubers was obtained with water extraction on water bath for 30 min.

Table 4 shows the dynamics of moving extractive substances and phenolic compounds to the extract during the topinmbur leaves extraction. As it can be seen from Table 4, during the first extraction of leaves about 39.0% extractive substances were moved to the extract and after three extractions 74.0% extractive substances were found in the extracts. In the following extractions the rate of moving of extractive substances to the extract decreased and after the fifth extraction it became slight.

Table 3. Determination of optimal regime of extraction of inulin from topinambur tubers

Output of Inulin (% of total content)	Conditions of extraction											
	Concentration of extragent (alcohol)				Temperature, °C				Duration of extraction, min			
	96°	50°	25°	0° (distilled water)	40	60	80	100	15	30	45	60
	83.5	89.7	96.3	101.3	84.6	88.7	93.0	101.8	87.8	101.2	93.4	90.2

Table 4. Dynamics of moving extractive substances and phenolic compounds to the extract during the extraction of topinambur leaves

Extraction	Extractive substances, % of total content	Phenolic compounds, % of total content	Phenolic compounds per extractive substances, %
First	38.8	30.0	17.8
Second	24,5	23.4	22.9
Third	10.9	12..0	25.5
Fourth	6.3	7.8	28.6
Fifth	4.1	6.0	32.7
Sixth	2.9	4.7	35.0
Seventh	2.0	2.7	35.0
Eighth	1.4	2.6	42.0
Ninth	1.2	2.1	39.5
Tenth	1.2	1.9	40.0

The data given in Table 4 show that in the first extract of topinmbur leaves phenolic compounds were found to be 17.8%. During the following extractions the content of phenolic compounds in the extracts gradually increased and reached maximum level at the eighth extraction (42.0%). In spite of the fact that the part of phenolic compounds in the extractive substances increased with the increase of the repetition factor of extraction, the main part of extractive substances moved to the extract during the first three extractions. So, it may be concluded that for the extraction of phenolic compounds from the topinambur leaves 3-fold extraction is enough.

Thus, topinambur tubers gathered in different regions of Georgia both in spring and autumn may be successfully used as raw materials for producing inulin and a multifunctional

biologically active food additive. It should be mentioned that both tubers (with high content of inulin) and leaves (with high content of phenolic compounds and accordingly high antioxidant activity) of topinambur should be used to obtain a multifunctional biologically active food additive while inulin can be produced only from topinambur tubers.

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საქართველოში ინტროდუცირებული მიწავაშლას შესწავლა ფუნქციური საკვები დანამატის მიღების მიზნით

აროშიძე ნ.[1], ომიაძე ნ. [2], მჭედლიშვილი ნ. [2], აბუთიძე მ. [2], კვესიტაძე გ.[2]

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რეზიუმე

გამოკვლეულია საქართველოში ინტროდუცირებული მცენარე მიწავაშლა ფუნქციური საკვები დანამატის მისაღებად. ნაჩვენებია, რომ მიწავაშლას ბოლქვები შეიცავს 92.0% ექსტრაქტულ ნივთიერებებს, მათ შორის 17.0% ინულინს. ამ მცენარის ფოთლები შეიცავს საკმაოდ დიდი რაოდენობით ფენოლურ ნაერთებს (12.0%) და პექტინის ნივთიერებებს (9.0%). მიწავაშლას როგორც ბოლქვები, ისე ფოთლები შეიცავს შაქრებს, 7.0 და 13.0% შესაბამისად. დადგენილია მიწავაშლას ბოლქვებიდან ინულინის და ამავე მცენარის ფოთლებიდან ფენოლური ნაერთების ექსტრაქციის რეჟიმები. ნაჩვენებია, რომ საქართველოს სხვადასხვა რეგიონში, მოყვანილი მიწავაშლა შეიძლება წარმატებით იქნას გამოყენებული ნედლეული ინულინისა და ფუნქციური ბიოლოგიურად აქტიური საკვები დანამატის მისაღებად.

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Biotechnology

INFLUENCE OF BIOSURFACTANTS ON SYNTHESIS AND STABILITY OF CELLULASES

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Abstract

Cellulase producer thermophilic strain – *Aspergillus versicolor* J1-5 was selected from the collection of micromycetes of Durmishidze Institute of Biochemistry and Biotechnology. The temperature and pH optimums of cellulolytic preparation of *Aspergillus versicolor* J1-5 were established. Effect of biosurfactants (ramnolipid – S_1, biocomplex – S_2, trehalolipid – S_3) on the synthesis of microbial cellulases, their enzymatic activity, thermostability was studied. Enrichment of nutrient medium with 0.01% of biosurfactant (S_2) caused enhancement of cellulolytic activity by 60%. Supplying of incubation medium with surfactants did not cause significant change in enzyme activity. After adding the biosurfactants stability of enzyme increased by 28%. Influence of biosurfactants on enzyme ability to deep hydrolysis of cellulose containing substrate (filter paper) forming low molecular reducing sugars, including glucose, has been investigated.

Key words: biosurfactant, cellulases, hydrolysis, thermostability.

Introduction

Manufacturing of enzyme preparations is a principal direction of recent biotechnology. Enzyme technologies are energetically sparing, cheap and ecologically safe. Hydrolytic enzymes like cellulases are lignocellulase-degrading ones. They are widely used in alcohol-, monosaccharide- and syrup-making technologies. Microscopic fungi are main producers of cellulases [Bhat, 2000].

The main problem preventing intensive application of enzyme technologies is expensiveness and low stability of enzyme preparations. One of the broadly used forms of enzyme stabilization is utilization of low-molecular substances, which increase in some extent the stability of enzymes against different extreme conditions (high temperature, marginal pH, high salinity).

Surfactants may be used as enzyme-stabilizing factor. They consist of amphipathic molecules and contain two functional groups – polar with hydrophilic head group, and non-polar with lipophilic tail. Biosurfactants may be both synthetic and natural. They are surface-active substances synthesized by living cells. From the environmental point of view biosurfactants attract an increasing attention due to their unique properties: low toxicity, biodegradability, and biological activities, compared to chemically synthesized counterparts. Biosurfactants enhance the solubilization and emulsification of hydrophobic substances and increase their availability for microbial degradation [Banat et al., 2000; Eliseev & Kucher, 2001]. Biosurfactants are widely used in oil, mining, chemical, pharmaceutical and cosmetic industries. They are applied as skin and hair

conditioners. In food industry we meet them as additives like lecitine, glycerine containing esthers of fatty acids. Biosurfctants diminish the dividing borders both in water solutions and carbohydrate mixtures. This feature is significant for raising the oil output and in degradation of emulsions [Ron & Rosenberg, 2001]. Five classes of biosurfactants are known for today: glycolipids (rhamnolipids, trehalolipids, sophorolipids), lipopeptides and lipoproteins (lychenisin, subtilisin, polymixin, viscosin, emulsan, liposan), lipopolysacharides (emulsan and xantane), fatty acids and phospholipids. For biosurfactants is characteristic selectivity and specific activity to high temperature, pH and ion concentration [Ron & Rosenberg, 2001; Desai & Banat, 1997].

The purpose of the study was to investigate the effect of biosurfactants on the synthesis and thermostability of microbial cellulases. Glycolipids – rhamnolipid (S_1), trehalolipid (S_3) and biocomplex (S_2) – the mixes of biosurfactants were used as testing materials. Glycolipids were selected according to their stability to high temperature. Their emulsifying properties are fully retained at 100°C under neutral or alkali pH during 2 hours.

Materials and Methods

The thermophilic strain *Aspergillus versicolor* J1-5 was selected from the collection of micromycetes of Durmishidze Institute of Biochemistry and Biotechnology. Submerged cultivation of micromycetes was performed in 750ml conic flasks, on a thermostated shaker (180-200rot/min), at 40°C during 96h. Composition of the liquid nutrient medium was following (%): microcrystalline cellulose – 0.1, $NaNO_3$ – 0.3, KH_2PO_4 – 0.2, $MgSO_4 \cdot 7H_2O$ – 0.05, corn extract – 1.5. 0.01%. The mixes of different biosurfactants were added to the nutrient medium to investigate the effect of biosurfactants. Dinitrosalycilic method [Adney & Baker, 2008] was used to determine the total cellulolytic activity of the cultural liquid. Filter paper (Wattman N1×1cm) was used as a substrate. Content of glucose was determined by glucoso-oxidase-peroxidase method [Rukhliadeva & Goriacheva, 1960]. To establish the extent of thermostability of cellulases the cultural liquid of the enzyme was placed at 63°C without any substrate for 40min. The initial activity was considered as 100%. Residual activity was expressed in per cents from the initial activity.

For the full hydrolysis filter paper (78g) was added by 5ml of 0.05M acetate buffer with pH4.5. Hydrolysis was conducted at 63°C during 48h. At the beginning of experiment surfactants in amount of 0.01% of the total volume were added into the incubation medium. The enzyme solution was added in amount of one unit, according to the activity. During intervals of time samples were taken and content of reducing sugars and glucose was determined following the standard method.

Results and Discussion

On the first step of investigation the optimal conditions for activity of the cellulolytic preparation of *Aspergillus versicolor* J1-5 were established (Table 1). Obtained results demonstrate that the preparation reaches its maximal activity while being incubated at 60°C and pH4.5-5. Enrichment of the nutrient medium with 0.01% surfactants induced increase of cellulolytic activity by about 60% (Table 2). Supplying directly the incubation medium with surfactants did not reveal significant effect on enzymatic activity.

Thermostability, or ability to operate at high temperature, is one of the significant features of enzyme preparation from the practical point of view. Thermo inactivation of the cellulolytic preparation of *Aspergillus versicolor* J1-5 was performed at 63°C, without substrate. The enzyme solution was added with biosurfactants in amount of 0.01% from the total volume (Table 3). According to obtained data it is clear that adding the incubation medium with S_1 and S_2

biosurfactants decreased the initial activity of the enzyme, but caused rise of its stability by 28%. Mostly effective appeared to be the biocomplex (S_2), which increased cellulase stability for 38%. Presumably biosurfactants join to particular sections of the enzyme molecule, and enhances enzyme's stability during the process of hydrolysis.

The extent of substrate's hydrolysis is another significant characteristic of hydrolyzing enzymes. Mostly effective appeared to be S_2 (extent of hydrolysis 40%). In cases of S_1 and S_3 amount of reducing sugars did not increase after 4-5h, similarly to the control, but content of glucose raised (Table 4). Adding of S_2 caused increase of reducing sugars during 24h. Presumably S_2 complex caused emulsification of the substrate, and formation of micelles; which served for the deeper hydrolysis of the substrate.

Table 1. Optimal conditions for activity of the cellulase of *Aspergillus versicolor* J1-5

temperature	40°C	45°C	50°C	55°C	60°C	65°C
strain	A FP un/ml	A FP un/ml	A FP un/ml	A FP un/ml	A FP un/ml	A FP un/ml
Aspergillus versicolor J1-5	0.35	0.35	0.4	0.4	0.5	0.35
pH	3.0	3.5	4.0	4.0	5.0	5.5
strain	A FP un/ml	A FP un/ml	A FP un/ml	A FP un/ml	A FP un/ml	A FP un/ml
Aspergillus versicolor J1-5	0.25	0.5	0.5	0.42	0.4	0.35

Table 2. Total cellulolytic activity of *Aspergillus versicolor* during submerged cultivation together with 0.01% of surfactants in the nutrient medium

Nutrient medium	K without addition	S_1 0.01%	S_2 0.01%	S_3 0.01%
A FP Aun/ml	0.5	0.75	0.83	0.8

Table 3. Influence of biosurfactants on thermostability of cellulase of *Aspergillus versicolor*

Incubation medium	Initial activity U/ml	Activity at 63°C after thermoinactivation, U/ml	Residual activity after thermoinactivation, %
K without addition	0.5	0.21	42
K + S_1	0.5	0.32	65
K+ S_2	0.4	0.32	80
K+ S_3	0.4	0.26	65

Table 4. Influence of biosurfactants on the degree of hydrolysis of filter paper by the cellulase obtained from *Aspergillus versicolor*. Experimental conditions: hydrolysis at 63°C; Substrate – filter paper, 78g; Acetate buffer – 5ml, pH4.5, 0.05M; Cultural solution of the enzyme – one unit

Incubation medium	Time of incubation											
	1h		2h		3h		4h		5h		24h	
	RS%	G%	RS%	G%	RS%	G%	RS%	G%	RS%	G%	Sh%	G%
K + S_1	8.7	2,6	12.8	3.5	16.1	3.8	22.2	6.4	24.4	8.3	24.4	12.0
K + S_2	12.2	3.8	17.3	4.5	21.2	4.8	25.0	7.7	28.2	9.4	40	19.2
K+ S_3	9.6	3.8	17.3	4.5	20.7	4.9	24.3	7.7	24.4	9.0	24.4	16.0
K	7.4	2.6	12.3	3.6	15.6	3.8	21.5	6.0	24.4	7.7	24.4	12.3

Table 5. Share of glucose (%) in reducing sugars obtained after the full hydrolysis of a filter paper

Nutrient medium	K without additions	S_1 0.01%	S_2 0.01%	S_3 0.01
share of glucose in reducing sugars, %	51	53	50	66

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ბიოსურფაქტანტების გავლენა ცელულაზების სინთეზსა და სტაბილობაზე

ქუთათელაძე ლ., ურუშაძე თ., ხვედელიძე რ., ალექსიძე თ.

საქართველოს აგრარული უნივერსიტეტის დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

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რეზიუმე

დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტის მიკრომიცეტების კოლექციიდან შერჩეულია თერმოფილური შტამი - *Aspergillus versicolor* J1-5. დადგენილია *Aspergillus versicolor* J1-5-ის ცელულაზური პრეპარატის პ და ტემპერატურული ოპტიმუმები. შესწავლილია ბიოსურფაქტანტების გავლენა მიკრობული ცელულაზების სინთეზზე, ფერმენტულ აქტივობაზე, თერმომედეგობაზე და ცელულოზური სუბსტრატის (ფილტრის ქაღალდი) ჰიდროლიზის ხარისხზე. დადგენილია, რომ ბიოსურფაქტანტების კომპლექსის დამატება საკვებ არეში იწვევს საერთო ცელულაზური აქტივობის ზრდას 60%-ით. უშუალოდ საინკუბაციო არეში ბიოსურფაქტანტების დამატებას არ ჰქონდა ეფექტი ცელულაზურ აქტივობაზე, მაგრამ ფერმენტის თერმომედეგობა გაიზარა 28%-ით. ბიოსურფაქტანტების კომპლექსის დამატებამ გამოიწვია ჰიდროლიზის პროცესის გახანგრძლივება და გლუკოზის გამოსავლის გაზრდა.

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MORPHOLOGICAL RELATIONSHIPS BETWEEN WILD AND CULTIVATED PEARS IN GEORGIA

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Abstract

214 individuals of 26 local, 9 introduced pear cultivars and 3 wild progenitor species of cultivated pear: *Pyrus caucasica* Fed.; *P. balansae* Decne. and *P. pyraster* (L.) Burgsd. have been evaluated by 27 morphological characters. 6 quantitative and 6 qualitative leaf and young shoot and 14 qualitative fruit descriptors have been used in multivariate statistical analysis. Leaf blade form have been analysed using Fourier's outline shape analysis method measuring 20 harmonics per leaf and 10 leaves per individual. 6 Principal Components have been used in Hierarchical Cluster Analysis and revealed close Euclidean similarity distances between 15 Georgian aboriginal pear cultivars and two wild species: *P. caucasica* (endemic of the Caucasus) and *P. balansae* (wild species native for Georgia). European wild pear - *P. pyraster*, was clustered with 9 introduced and 11 Georgian pear cultivars. The results of this study have shown that some local pear cultivars of Georgia are directly domesticated from the native wild pear species - *P. caucasica* and *P. balansae*. The other local cultivars might be obtained due to selective works by breeding of local landraces with introduced cultivars from different countries in historically different periods.

Key words: Domestication, Georgia, Caucasus, morphometry, pear cultivars, *Pyrus*.

Introduction

Many pear cultivars occur in Georgia from pre-historic period [Javakhishvili, 1930] indicating the early domestication event of this cultivated fruit tree. In total, 11 species of wild pear occur in Georgia [Kutateladze, 1980]. They are distributed in different regions, what is caused by the variable geographical relief and habitat diversity of the country. *Pyrus caucasica* Fed., the endemic species of the Caucasus is most widespread among the wild pears of Georgia and is considered as main progenitor species of local pear cultivars [Khomizurashvili, 1973]. Moreover, *P. caucasica* and *P. pyraster* (L.) Burgsd. are regarded as the main wild progenitors, from which the cultivated European pear (*P. communis* L.) has probably evolved [Zohary and Hopf, 2000; Volk et al., 2006; Yamamoto and Chevreau, 2009]. In modern classification system of the genus *Pyrus*, *P. caucasica* and *P. pyraster* are demoted to rank of subspecies of European pear *P. communis* [Browicz, 1993]. However, Caucasian wild pear is considered as separate species by many authors [Grossheim, 1946; Ketskhoveli, 1960; Tuz, 1974; Brezhnev and Korovina, 1981; Bläsing, 2004] based on arguments that the differences are not only morphological features, but as well separate geographic areas of distribution.

According to the literature data [Khomizurashvili & Eristavi, 1939, 1941; Khomizurashvili, 1973; Likhonos et al., 1983] introduced cultivars of pear from Europe appeared in Georgia

at the end of 19th century from Russia and before there were existed only the local cultivars. However, we assume that the process of cultivar introduction have to start much earlier, as Georgia had cultural contacts to Asian and European countries since antique period. It is also remarkable that Greece is considered as first provider of selective cultivars of pear to the ancient world [Jackson, 2003] and earliest relations between Georgia and Greece should be considered as possible way of introduction of European pear cultivars in the Caucasus.

According to the classification system by N. Khomizurashvili [1973] the Georgian pear cultivars are divided into four groups: 1) 'Gulabi'; 2) 'Panta Mskhali'; 3) 'Kalos Mskhali'; and 4) 'Khechechuri'. The names of each group represents name of a cultivar, which is considered as typical representative of the group. According to the literature data [Khomizurashvili, 1973], the last three groups are originated by direct domestication of wild Caucasian pear in Georgia, however, this idea is not confirmed by statistical methods of taxonomic identification and relationships among taxa. Therefore, we decided to conduct comparative morphometric study of cultivars recently occurred in Georgia and reveal their relationship to local wild pear species. The results have to determine local cultivars originated by direct domestication events in ancient time and discriminate from cultivars, which will have relationships with other wild species - *P. pyraster* or *P. pyrifolia,* considered as wild ancestors of European and Far East pear cultivars respectively.

The aim of the present study was: 1) to determine morphological characteristics of leaves, young shoots and fruits differentiating local and introduced pear cultivars of Georgia; and, 2) to reveal the relationships between cultivars and wild ancestor species of pear by statistical methods used in plant morphology.

Materials and Methods

Field sampling: A total of 214 wild and cultivated pear trees have been sampled in natural habitats, living collections and peasant grounds in different regions of Georgia. Wild pear species were determined according to Kutateladze [1980]. The wild pear accessions evaluated in this study consisted of Caucasian endemic *P. caucasica* Fed. (=*P. communis* subsp. *caucasica* (Fed.) Browicz; N=100), *P. balansae* Decne. (= *P. communis* L.; N=8) and *P. pyraster* (L.) Burgsd. (=*Pyrus communis* L. subsp. *pyraster* (L.) Ehrh.; N=3), which has been obtained from Germany, Hessen, in surrounding of v. Erda.

81 individuals of 26 Georgian local (LC) and 22 individuals of 9 introduced (IC) cultivars (total 103 individuals of 35 cultivars) have been sampled. ICs were obtained from the collection of the Institute of Horticulture, Viticulture and Oenology (IHVO), v. Skra, Gori distr., Georgia; LCs were sampled in the collection of aboriginal cultivars of Biological Farming Association "ELKANA" in v. Tsnisi, Akhaltsikhe distr., Georgia. Many local cultivars are collected in peasant grounds in different regions of Georgia. The names of local Georgian pear cultivars collected in villages have been identified according to N. Khomizurashvili [1973]. The name of cultivar 'Khinos mskhali' was not identified and we used name of the village where it was collected. Names and the country of origin of introduced pear cultivars have been identified according to N. Khomizurashvili and E. Eristavi [1939, 1941].

10 mature leaves and 5 fruits per individual have been collected from randomly selected plants in each location from August till the end of September 2009 and 2010. The samples have been obtained only from adult trees with diameters at the breast height ranging from 0.20 m to 0.90 m. Coordinates and elevation have been collected by GPS "E*trex*", Legend HCx, Garmin. The habitat type and plant community have been identified. Leaves have been kept in herbarium and were digitalized by Nikon Coolpix 5500 camera for morphometric and landmark analysis.

Morphological analysis: The individuals were evaluated by 27 morphological traits, which included one landmark analysis data (20 harmonics per leaf), 12 leaf and shoot descriptors and 14 fruit descriptors. Morphological characters have been taken as recommended by UPOV [2000] for *P. communis* and Voltas et al. [2007], which delimited differences between wild and cultivated taxa of the genus *Pyrus* based on morphometric analysis. 20 harmonics per leaf and 10 leaves per individual have been analysed using Fourier outline shape analysis method for the measurement of form of the leaf blade in studied taxa. Leaves have been analysed as digital images by free landmark analysis software package "Shape 1.3" [Iwata, 2006].

Six traits of leaves were quantitative, 6 qualitative for leaves and 10 for fruits, 4 traits were seasonal characters of fruit appearance and maturation. The quantitative traits (leaf blade and petiole length and width) were measured to an accuracy of 0.1 mm using a micrometer. Two ratios involving quantitative measurements defined two additional leaf traits (leaf blade width to length and blade length to petiole length). The length and width of the fruit and the stalk despite its quantitative nature was transformed into qualitative ordinal variables. The fruit qualitative characters have been analysed by descriptors [UPOV, 2000]. The stone cell amount and leaf margin shape have been determined using stereo microscope Stemi DV4 (Karl Zeiss, Germany). Two values of a tree were evaluated in the field: crown shape and thorn amount. Seasonal traits have been determined in the field based on the information obtained from the local population and literature data.

Statistical analysis: Quantitative characters of morphological traits by each descriptor were recorded as average value per tree. Quantitative traits of leaf morphological characters have been selected by high F values and significance ($p<0.05$) calculated by One-Way ANOVA procedure. Qualitative traits of leaves and fruits were selected by the test of independence (Chi-square). Four traits of seasonal characters of fruit appearance and maturation and 2 shoot descriptors have been rejected from analyses. In total 21 morphological traits have been used during multivariate analysis. The Principal Components Analysis (PCA) was based on the covariance matrix of the coefficients and don't on the correlation matrix, because coefficients with small variance and covariance values of harmonics of Fourier coefficients are generally not important for explaining the observed morphological variations of leaf blade shape revealing a pattern of variation that is consistent with the distribution rate of wild pear species and cultivars. The factors obtained by PCA were used for conducting of the Hierarchical Cluster Analysis (HCA) to specify the distance or similarity measure to be used in clustering with the Ward's method as amalgamation rules [Ward 1963]. This method uses an analysis of variance approach to evaluate the distances between clusters. This attempts to minimize the Sum of Squares (SS) of any two (hypothetical) clusters that can be formed at each step. Distance measure interval is Euclidean distance computing distances between objects in a multi-dimensional space.

Statistical analyses have been performed using the software packages SPSS v.13.0 for Windows [SPSS Inc., 2004] and Statistica 6.0.

Results and Discussion

PCA analysis of 20 harmonics, 6 quantitative leaf traits and 14 qualitative descriptors of leaf, shoot and fruit revealed the first six PCs explaining 87.6 % of the variance (Table 1). 15 PCs are needed to account for 98.77 % of the overall variance. The first 6 PCs have been used to conduct HCA. The highest loadings on the 1st PC correspond to characters related to the quantitative leaf traits. The variables with the highest loadings on the 2nd PC are leaf blade base form, leaf length and fruit flesh colour. And the highest loadings on the 3rd PC are the leaf blade

width and ratio between the blade length and width. The average for taxon was calculated for each of 6 PCs.

Table 1. Results of PCA based on 21 morphological traits (20 harmonics, 6 quantitative leaf descriptors and 14 qualitative descriptors of leaf, shoot and fruit). Extraction sums of squared loadings of the first 6 PCs. (N=214).

Component	Total	% of Variance	Cumulative %
1	13.604	47.137	47.137
2	4.655	16.131	63.268
3	2.759	9.561	72.830
4	2.018	6.992	79.822
5	1.271	4.405	84.227
6	0.973	3.372	87.599

The relationships between the 35 cultivars and 3 wild species of pear - *P. balansae, P. caucasica* and *P. pyraster* are reflected in the HCA dendrogram of Euclidean Distance with the Ward's method as amalgamation rules (Fig. 1). The taxa in the dendrogram are clustered into two main groups (A and B). The group A includes *P. caucasica* and *P. balansae* from wild species and 15 Georgian LCs. The second group B contains *P. pyraster* and 11 LCs and 9 ICs.

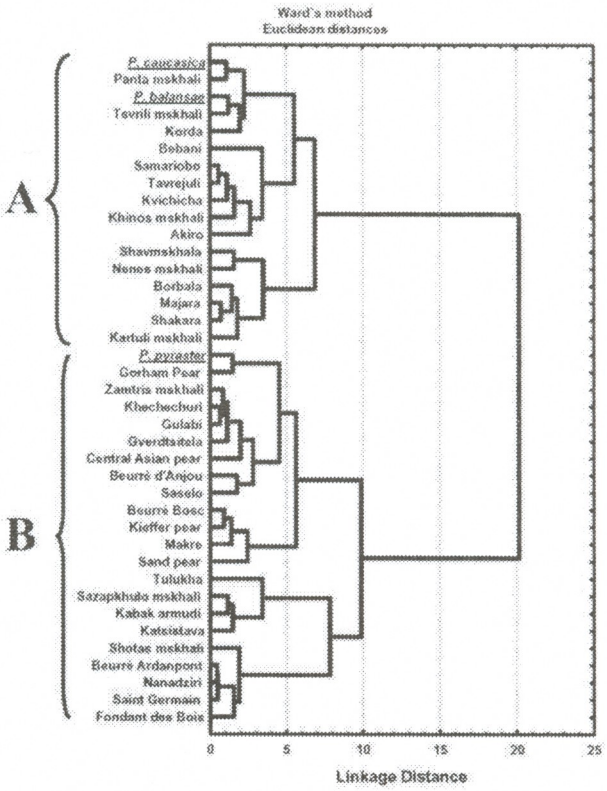

Fig. 1. HCA dendrogram of Euclidean distance with the Ward's method showing the relationships between the 35 cultivars and 3 wild species of pear based on 20 morphological traits of leaf, shoot and fruit and 20 landmark harmonics of mature leaf; the taxa in the dendrogram are clustered into two main groups - A and B. (N=214).

Table. 2. Classification of Georgian LCs and ICs based on morphological similarity distance with wild pear species - *P. balansae, P. caucasica* and *P. pyraster.* 1st group contains 15 LCs related with the wild Georgian pear species - *P. caucasica* and *P. balansae;* 2nd group is represented by 11 LCs related with European wild pear - *P. pyraster* and ICs; 3rd group containes 9 ICs. (N=35).

N	Cultivar name	Georgian common name	Locality	Progenitor species	Country of origin
Group 1: Local cultivars related to *Pyrus caucasica* and *P. balansae*					
1.	Panta mskhali	პანტა მსხალი	Georgia	*P. caucasica*	Georgia
2.	Tsvrili mskhali	წვრილი მსხალი	Svaneti	*P. balansae*	Georgia
3.	Korda	კორდა	Meskheti	*P. balansae*	Georgia
4.	Bebani	ბებანი	Svaneti	*P. caucasica*	Georgia
5.	Samariobo	სამარიობო	E-Georgia*	*P. caucasica*	Georgia
6.	Tavrejuli	თავრეჯული	Meskheti	*P. caucasica*	Georgia
7.	Kvichicha	კვიჭიჭა	W-Georgia*	*P. caucasica*	Georgia
8.	Khinos mskhali	ხინოს მსხალი	Adjara	*P. balansae*	Georgia
9.	Akiro	აყირო	Imereti	*P. caucasica*	Georgia
10.	Shavmskhala	შავმსხალა	Georgia	*P. caucasica*	Georgia
11.	Nenes mskhali	ნენეს მსხალი	W-Georgia*	*P. caucasica*	Georgia
12.	Borbala	ბორბალა	Kartli	*P. caucasica*	Georgia
13.	Machara	მაჭარა	Meskheti	*P. caucasica*	Georgia
14.	Shakara	შაქარა	Meskheti	*P. caucasica*	Georgia
15.	Kartuli mskhali	ქართული მსხალი	Imereti	*P. caucasica*	Georgia
Group 2: Georgian LCs related to *Pyrus pyraster* and ICs					
16.	Zamtris mskhali	ზამთრის მსხალი	Georgia	*unknown*	Georgia
17.	Khechechuri	ხეჭეჭური	Georgia	*unknown*	Georgia
18.	Gulabi	გულაბი	Georgia	*unknown*	unknown
19.	Gverdtsitela	გვერდწითელა	Georgia	*unknown*	Georgia
20.	Saselo	სასელო	Meskheti	*unknown*	Georgia
21.	Makre	მაქრე	Samegrelo	*unknown*	Georgia
22.	Sazaphkhulo mskhali	საზაფხულო მსხალი	Georgia	*unknown*	Georgia
23.	Tulukha	თულუხა	Kartli	*unknown*	Georgia
24.	Katsistava	კაცისთავა	W-Georgia*	*unknown*	Georgia
25.	Nanaziri	ნანაზირი	Meskheti	*unknown*	Georgia
26.	Shotas mskhali	შოთას მსხალი	Kartli	*unknown*	Georgia
Group 3: Introduced cultivars					
27.	Central Asian pear	შუააზიური მსხალი	Kartli	*P. communis*	Central Asia
28.	Beurré d'Anjou	ანჟუის სილამაზე	Georgia	*P. communis*	France
29.	Beurré Bosc	ბერე ბოსკი	Georgia	*P. communis*	France
30.	Kiffer pear	კიფერის თესლნერგი	Georgia	*P. communis* 'Bartlett' x *P. pyrifolia* 'Sand pear'	United States
31.	Sand pear	ირანული მსხალი	W-Georgia*	*P. pyrifolia*	Far East Asia
32.	Kabak armudi	ყაბაღ არმუდი	Meskheti	*P. communis*	Turkey/Georgia
33.	Beurré Ardanpont	ბერე არდანპონი	Kartli	*P. communis*	Belgium
34.	Saint Germain	სენ-ჟერმენი	Georgia	*P. communis*	France
35.	Fondant des Bois	ტყის სილამაზე	Georgia	*P. communis*	Belgium

* E - East; W - West.

These results are used to classify pear cultivars distributed in Georgia to three different groups (Table 2): 1) 15 local cultivars form cluster A are related with Caucasian wild pear (*P. caucasica*) and Balansae's pear (*P. balansae*); 2) 11 LCs from cluster B are historically Georgian cultivars occurring in Georgia long time, but according to obtained results they are related to wild *P. pyraster* and introduced cultivars of *P. communis*. 3) 9 cultivars from cluster B are introduced from abroad or originated in breeding stations in Soviet time.

According to HCA's results, pear cultivars analysed in this study are clustered into two groups (Fig. 1): The first group A contains LCs related to *P. caucasica* and *P. balansae* by 21 morphological traits of leaves and fruits. Especially close Euclidean similarity distance is revealed between wild Caucasian pear - "Panta" in Georgian and a cultivar named as 'Panta mskhali', which confirms etymological and taxonomic similarity within these taxa. *P. balansae* shows very close similarity distance with 'Tsvrili mskhali' and 'Korda'. Very closely related group of LCs to wild Georgian pears contains: 'Bebani', 'Samariobo', 'Tavrejuli', 'Kvichicha', 'Khinos mskhali' and 'Akiro'. The other group: 'Shavmskhala', 'Nenes mskhali', 'Borbala', 'Majara', 'Shakara' and 'Kartuli mskhali', is more distanced from wild pears, but located in the same cluster. We assume that these LCs must have originated by direct domestication of wild pears in Georgia (Table 2).

The second cluster B (Fig.1) contains ICs of pear originated in European countries and some old Georgian cultivars. The group B reveals relationship with wild pear - *P. pyraster,* which is distributed in Europe and does not reach Georgian territory. The area of distribution is up to the middle of Turkey. The most cultivars from intermediate group B are more widespread in Georgia than the LCs originated by direct domestication of wild pears from group A. According to the literature data [Sharashenidze, 1956; Nadiradze, 1966; Tsikvadze and Isakadze, 1969], two local pears 'Gulabi' and 'Khechechuri' are the most widespread from all local pears of Georgia and there are two or more varieties of them in each localities of the country. Moreover, Georgian name of cultivar 'Gulabi', which is also used as the name of local pear group in classification of N. Khomizurashvili [1973], means 'Pear' (گلابی 'golabi') in Persian. We suggest that LCs from cluster B (Fig.1) might have appeared in Georgia very long time ago and were improved by local population using breeding procedures.

According to A. Fedorov [1943], leaf margin shape is the main morphological trait that differentiates Caucasian pear (*P. caucasica*) from European pear (*P. communis*). This theory was proved by the statistical analysis of the collected samples for the present study. Nowadays, *P. pyraster* is considered as the wild pear of Europe and cultivars are named as *P. communis* (Stephan et al., 2003; Yamamoto and Chevreau, 2009). 'Communis' or the 'Common pear' group of cultivars has become the name of the cultivated pears of Europe, however, the structure and the diversity of the wild and cultivated pears of this group is not studied in details and needs further genetic and molecular investigations.

Thus, the results of this study have shown that some LCs of Georgia are directly domesticated from the native wild pear species - *P. caucasica* and *P. balansae.* The other LCs might be obtained due to selective works by breeding of local landraces with introduced cultivars from different countries in historically different periods. The molecular study of these taxa will clear in more details origin of these cultivars.

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მორფოლოგიური ურთიერთკავშირი საქართველოში გავრცელებულ კულტურულ და ველურ მსხლებს შორის

ასანიძე ზ, ახალკაცი, მ.

ეკოლოგიის ინსტიტუტი, ბოტანიკის ინსტიტუტი, ილიას სახელმწიფო უნივერსიტეტი

(მიღებულია 13.06.2011)

რეზიუმე

შესწავლილია მსხლის 26 ქართული, 9 ინტროდუცირებული ჯიშის და კულტურული მსხლის 3 ველური წინაპარი სახეობის: *Pyrus caucasica* Fed.; *P. balansae* Decne. და *P. pyraster* (L.) Burgsd., 214 ინდივიდი. 27 მორფოლოგიური ნიშანი გამოყენებულია მულტივარიაციული სტატისტიკური ანალიზისთვის. მათ შორისაა, ფოთლის და ახალგაზრდა ყლორტის 6 რაოდენობრივი და 6 ხარისხობრივი მახასიათებელი, ნაყოფის 14 ხარისხობრივი დესკრიპტორი და ფოთლის ფორმა, რომელიც ყოველი ინდივიდისთვის გამოთვლილია, როგორც ფურიეს კონტურის ფორმის ანალიზის მეთოდით გაზომილი 20 ჰარმონიკის და 10 ფოთლის საშუალო მაჩვენებლი. 6 პრინციპული კომპონენტი იქნა გამოყენებული იერარქიული კლასტერული ანალიზისთვის და ევკლიდეს მსგავსების ახლო მანძილი გამოვლინდა 15 ქართულ აბორიგენულ მსხლის კულტივარსა და ორ ველურ სახეობას შორის: *P. caucasica* (კავკასიის ენდემი) და *P. balansae* (საქართველოს ადგილობრივი სახეობა). ევროპული ველური სახეობა - *P. pyraster*, გაერთიანდა 9 ინტროდუცირებულ და 11 ქართულ კულტივართან ერთად. კვლევის შედეგები მიუთითებენ, რომ მსხლის ზოგიერთი ქართული კულტივარის დომესტიკაცია განხორციელებულია პანტის და ბალანსეს მსხლის პირდაპირი მოშინაურების გზით. მსხლის ზოგიერთი ქართული კულტივარი კი შესაძლებელია წარმოშობილი იყოს ადგილობრივი და ინტროდუცირებული ჯიშების სელექციური ჰიბრიდიზაციით სხვადასხვა ისტორიულ პერიოდში.

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NATURAL FEATER GRASS STEPPES (STIPETA PENNATAE) ON THE TERRITORY OF NATIONAL BOTANICAL GARDEN OF GEORGIA

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Abstract

The structure of feater grass community (Stipetum pennatae) spread wildly in the National Botanical Garden of Georgia (Tbilisi) has been studied. Its phytocoenological-floristic characteristics are given. This considered community has not been grazed for a long time that has reflected on its structure. Particularly great amount of litter is accumulated and as a result, an ephemeral sinuzia is developed weakly. Accordingly, portion and coenetic role of an annual plant is much reduced, floristic composition is also lowered. According to the floristic analysis it is established that the studied community, in which creation the role of Caucasian species are important, is in florogenesic relationship, on one hand with Southwest Asia and on the other hand with Eurasian steppes. A Mediterranean influence occurs also.

Key words: plant community, phytocoenological characteristics, floristic composition, geographical range type, life forms.

Introduction

National Botanical Garden of Georgia is situated on the downstream of the river Tsavkistskali gorge, between Tabori and Sololaki ridges. Semiarid climate with moderate cold winter and warm summer is typical. Average annual temperature is 12.6°, mean annual precipitation is 554 mm (Fig. 1). Most of the precipitation comes in spring and in the first half of summer, and the least of the precipitation – in winter [Kordzakhia M., 1961; Hand Book on Climate of USSR, 1967, 1970].

An important part of the territory of the garden (about 47%) is occupied by the natural landscape, which is represented mainly by hemixirophilous vegetation (steppe, shrubbery, xerophilous complex, etc.). The aim of our research was to study the communities of feater grass steppe (Stipeta pennatae) spread on the territory of the garden; to establish their phytocoenological-floristic structure and florogenesic relationship.

Materials and Methods

Research of the phytocoenological-floristic data was made by the route method in 2009-2011. 38 geobotanical surveys were made (the space of each plot -1 m^2). During the surveys, we

used traditional geobotanical methods [Shennikov, 1964; Rabotnov, 1983; Vasilevich, 1985]. Species richness and frequency of occurrence were studied by the methods of A. Korchagin [Korchagin, 1964], Brown-Blanquet [Braun-Blanquet, 1951] and V. Ponyatovskaya [Ponyatovskaya, 1964]. During establishing the geographical range types, we followed the methods of R. Kamelin (1973), N. Portinier (2000a, b) and R. Gagnidze (20004). Life forms of the plants are separated on the basis of classification of C. Raunkiaer (1934) and I. Serebriakov (1964). Plant nomenclature follows R. Gagnidze (2005).

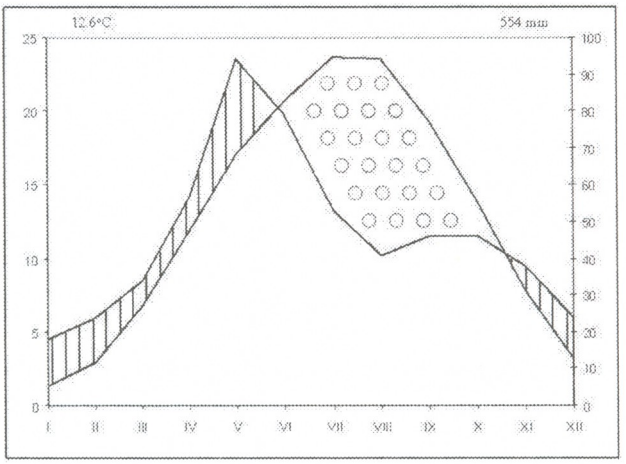

Fig. 1. Climadiagram of National Botanical Garden of Georgia

Results and Discussion

Areal. Relief. Substrat. In the National Botanical Garden of Georgia feater grass communities are spread on the north slope of Tabori ridges (470-520 m amsl). Coenoses are developed on North, North-West, East and North-East-facing slopes. Slope inclination is mainly around 30-35^0. Skeletal cinnamonic soil is developed there.

Growth. Growth of plants is going almost during the whole year. The period of its maximal development is in May-June and coincides with the maximum of atmosphere precipitation. In July-August-September most of the plants are dry, which is determined by drought period. In autumn some of the plants are in blossom. In winter, some plants winter in the form of rosette cropped up in autumn and continue their vegetation in spring. Such phenology is in compliance with the seasonal changeability of climate during the year.

Coverage. Projectional coverage of plants (living and dead cover together) is 80-100%. The cover of alive mass of vascular plants is 40-65%, moss cover is mostly around 45-60%. As the plots are reserved and have not been grazed for a long time, the great amount of detritions occur (especially the remains of the last year's dry leaves of *Stipa pennata* and other perennial grasses), and accordingly, the coverage of the litter is high, and around 40-70%.

Layer structure. At a maximal developing period (May-June) coenoses structure has 4 layers. I layer is mostly 80-90 cm height, II layer – 30-60 cm, III layer - 5-25 cm. The coverage of the living mass of vascular plants reduces from the top to the bottom according to the layers. The cover of I layer varies between 25-40 (45)%, II layer - from 1-2% to 30%; III layer - from some of the individuals (+) to 20%. It is important, that in most cases, an important role in creation of III layer belongs to dwarf semi-shrubs and less to the leaves of undeveloped individuals of annual

plants. Unlike from I layer, II and III layers are characterized by their nonstability of height and projectional coverage. According to each plot, their indicator varies hard. IV layer is created by the moss cover.

Synuzium structure. The following main synuzia are developed in the community: 1. turfing grasses, 2. perennial grass and forbs, 3. ephemers, 4. moss. From these designated synuzia, only the ephemerous synuzia is expressed too weakly. We do not meet ephemers on some plots or they are represented only by some individuals of 1-2 species and hence, synuzia is not formed at all. In semiarid climate and non-equal precipitation distribution conditions, such poor forming of ephemeral synuzia is determined by existence of powerful layer of litter, which brakes the spreading its seeds. At the same time small ephemerous plants have no means of normal sprouting out and development.

On the separated plot we meet hemixerophilous dwarf semi-shrub synuzia.

Sodding. Sodding level of the coenoses is around 15-35%.

Frequency of occurrence. 94 species of vascular pants were registered in the 38 surveyes. Among them only few of them (13.8% - 13 species) are characterized by high and average constants (frequency of occurrence 40-100%). They are: *Stipa pennata* (frequenvy of occurrence - 100%), *Rumex tuberosus* (92%), *Koeleria cristata* (81.5%), *Galium verum* (63%), *Melilotus neapolitanus* (60.5%), *Phleum phleoides* (58%), *Scorzonera biebersteinii* (52.5%), *Onobrychis cyri*, *Pterotheca sancta* (47.5-47.5%), *Bothriochloa ischaemum* (42%), *Allium atroviolaceum*, *Pimpinella aromatica*, *Potentilla recta* (40-40%). Most of the species have very low indicator of frequency of occurrence (Fig. 2) and they can be belonged to the casual species. Among the constant species there are only three therophyts (2 - annual, 1 - biennial), 2 - geophyts and most of them (8 species) are hemicriptophyts. Small amount of therophyts in the constant species can be explained by the weak development of ephemerous synuzia.

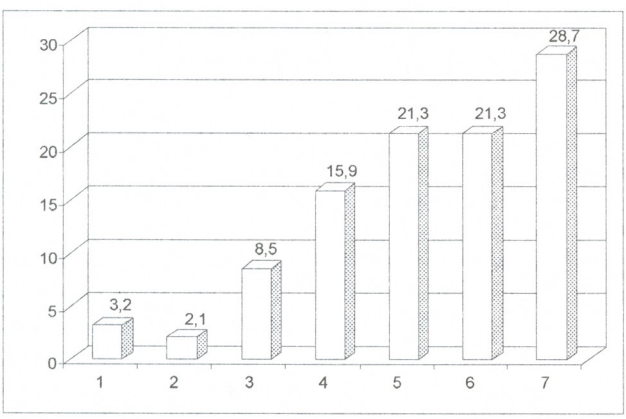

Fig. 2. Correlation of species (in-%) according to the frequency of occurrence
Frequency of occurrence: 1. 80-100%, 2. 60-79%, 3. 40-59%, 4. 20-39%, 5. 10-19%, 6. 5-9%, 7. 0.1-4%

Species richness. On average 16 species are registered on 1 m^2. Such indicator is too low for the plant community of steppe. As it is seen from the diagram (Fig. 3) amount of hemicriptophyts is higher than the amount of the other life form. Low richness of annual plants is especially important (2.2 species per 1 m^2). It is also significant that in 6 of 38 plots annual plants are not registered at all, in 10 plots only one species is fixed, and in 6 plots we met 2 species. Such low participation of annual plants is a result of weak development of ephemerous synuzia. This

determines comparatively low common indicator. Richness of shrubs, dwarf semi-shrubs and biennial plants per 1 m^2 is in standard limits as the steppe vegetation is characterized.

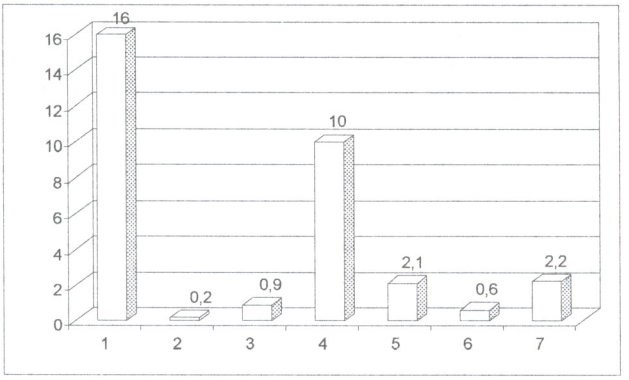

Fig. 3. Species richness (per 1 m^2)
1.General, 2 Shrubs, 3. Semi-shrubs & draft semi-shrubs, 4. Perenial grasses (hemicriptophiytes), 5. Perennial grasses (geophytes), 6. Biennial plants, 7. Annual plants.

Coenetic role of species. Most of the species are characterized by the unstable indicators of projectional coverage. According to each plot, projectional coverage and correspondingly coenetic role strongly vary (from one individual to some %). Also, development in groups and high projectional coverange of rare and casual species is noticed in some plots. Projectional coverage (minimal and maximal) of those plants whose frequency of occurrence is more than 20% is given below: *Stipa pennata* - 27-57%, *Koeleria cristata* - from some individuals (+) to 8%, *Galium verum* - from + to 14%, *Melilotus neapolitanus* - from + to 10%, *Phleum phleoides* - from + to 20%, *Scorzonera biebersteinii* - from + to 9%, *Onobrychis cyri* - from + to 8%, *Pterotheca sancta* - +, *Allium ateroviolaceum* - +, *Pimpinella aromatica* - from + to 6% *Potentilla recta* - from + to 4%, *Teucrium polium* - from +to 6%, *Zosima orientalis* - from + to 8%, *Allium rupestre* - +, *Festuca valesiaca* - from + to 8%, *Carex sp.* - from + to 7%, *Cleistogenes bulgarica* - +, *Medicago caerulea* - from + to 7%, *Bothriochloa ishaemum* - from + to 10%, *Dactylis glomerata* - from + to 5%, *Thalictrum collinum* - from +to 5%, *Echinops sphaerocephalus* - from + to 3%, *Gagea chlorantha* - from +, *Scutellaria orientalis* - from + to 8%, *Stachys atherocalyx* - from + to 9%, *Thymus coriifolius* - from + to 9%. From these data it is seen, that perennial forbs are distinguished by the comparatively high coenetic role and from annual plants only *Melilotus neapolitanus* occur.

Floristic composition. 94 species of vascular plants were registered, that belong to 79 genera and 27 families.

In floristic spectrum the leading families by the composition of genera are: 1. Poaceae - 16 genera (20.2%), 2. Asteraceae - 13 genera (16.5%), 3. Apiaceae - 7 (8.9%), 4. Lamiaceae - 6 (7.6%), 5-6. Fabaceae, Brassicaceae - 5-5 (6.3-6.3%), 7. Caryophyllaceae - 4 (5.1%), 8-10. Dipsacaceae, Polygonaceae, Rubiaceae - 2-2 (2.5-2.5%). 78.4% (61 genera) of total genera comes for their part. The rest 17 families are represented by one genus.

The leading families by the quantity of species are: 1. Poaceae - 16 species (17.1%), 2. Asteraceae - 14 (14.9%), 3-4. Fabaceae, Lamiaceae - 8-8 (8.5-8.5%), 5. Apiaceae - 7 (7.4%), 6. Brassicaceae - 5 (5.3 %), 7-9. Alliaceae, Caryophyllaceae, Rubiaceae - 4-4 (4.3-4.3%), 10. Euphorbiaceae - 3 (3.2%). 1-5 families consist of 56.4 % (53 species) of floristic composition of the community, and on the first 10 families comes 77.7 % (73 species). 4 families (Dipsacaceae, Liliaceae, Polygonacea, Scrophulariaceae) are represented by 2-2 species, and other 13 families –

by one species. As it is seen from the represented spectrum, the portions of the leading families are quite high.

Except plants, there was one by one species of lichen registered in two plots [*Cladonia furcata* (Huds.) Scharad. & *C. convoluta* (Lam.) Andrs.].

Geographical range types. Caucasian geographical range type is represented the most numerously – 19 species (20.2%), from which 9 are Caucasian and 1- Georgian endemic. So, endemism of floristic composition of the studied community is 10.6 %, which must be considered as a high indicator. Also Caucasian-Southwest Asian species are numerous - 13 species (13.8%). Portion of species connected with the Eurasian steppe areal is high. Those species are: Caucasian-Eurasian steppe (8 species), Mediterranean-Southwest Asian-Eurasian steppe (5), Mediterranean-Eurasian steppe (4). Beside them Mediterranean-Southwest Asian-Pontian (3) And Southwest Asian-Caucasian-Pontian (1) species can be considered. Common portion of these species is 22.3% (21 species). Influence of Mediterranean and Southwest Asian floristic centers is mainly expressed by the participation of Mediterranean-Southwest Asian (7 species), Mediterranean-Southwest Asian-Turanian (2), European-Mediterranean-Southwest Asian (2) and European-Mediterranean-Southwest Asian-Middle Asian (1) species. Their common portion is 12.8% (12 species). Participation of European-Mediterranean (6 species; 6.4%) also emphasize the influence of Mediterranean, and Southwest Asian floristical centre is emphasized by the great number of Caucasian-Southwest Asian. Directly Mediterranean is represented by 2 species, but Southwest Asian-Turanian geographical range type - by one species.

There were 8 Palaearctic species (8.5%) registered in the community. Except them we meet plants European-Ancient Mediterranean (4 species), Ancient Mediterranean (2), Palaearctic (3), Caucasian-Middle Asian (1) and European-Caucasian (1) of geographical range types.

Such proportion of geographical range types indicates, that studied feater grass community, in which creation local (Caucasian) species role is important, is in florogenesic relationship with on the one hand - Southwst Asia, and on the other hand with Eurasian steppe. There is a Mediterranean influence also.

Life forms. In life forms spectrum (Fig. 4) hemicryptophytes dominates, which amount is rather more than that of other life forms. Proportion of hemicryptophyte and therophytes (annual plant and biennial plant) is the most important. It is seen that portion of hemicryptophytes is two times more than therophytes. Such proportion, in our opinion, is not typical and it is not in correspondence with the physical geographical conditions of the Botanical Garden. Such difference is connected with the weak development of ephemeral sinuzia.

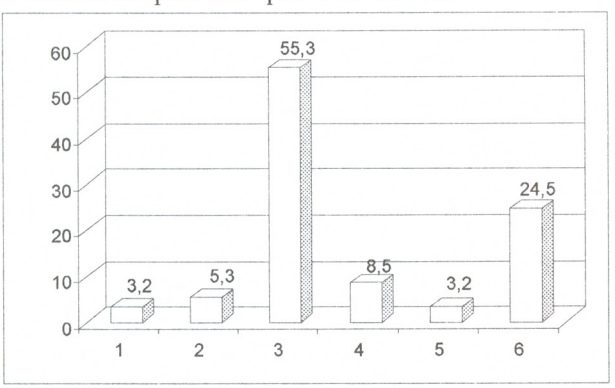

Fig. 4. Proportion (%) of life forms

1. Shrubs, 2. Semi-shrubs & draft semi-shrubs, 3. perennial herbaceous plants (hemicryptophytes), 4. perennial herbaceous plants (geophytes), 5. Biennial plants, 6. Annual plants.

From these represented data it is seen that total reservation cause important changes of vegetation structure. Floristic composition is reduced either. Similar results are in the yellow bluestem formation (Bothriochloeta ischaemum) cases of Shida Kartli [Lachashvili, et al., 2010].

The list of vascular plants registered in the community is given in Table 1.

Table 1. List of the vascular plants registered in the community

Family	Species	Life form	Chorotype	Frequency of occurrence (%)
Dicotyledoneae				
Apiaceae	*Astrodaucus orientalis*	Th	S.W. As.-Cauc.-Pon.	2.5
	Bupleurum exaltatum	H	Cauc.-S.W. As.	10.5
	Daucus carota	Th	Eur.-Anc. Med.	2.5
	Eryngium campestre	H	Eur.-Med.	8
	Pimpinella aromatica	Th	Cauc. (eastern Cauc. endemic)	40
	Seseli grandivittatum	Th	Cauc. (S. Cauc. endemic)	16
	Zosime orientalis	H	S.W. As.-Tur.	37
Asteraceae	*Achillea neilreichii*	H	Med. (E. Med.)	18.5
	Centaurea ovina	H	Cauc. (with N. & N.-W. Ir. irradiation)	5
	Centaurea reflexa	H	Cauc. (with Arm.-Kurd. irradiation)	13
	Chondrilla juncea	H	Med.-S.W. As.-Euras. step.	18.5
	Echinops sphaerocephalus	H	Med.- Euras. step.	21
	Galatella dracunculoides	H	Med.-S.W. As.-Euras. step.	8
	Hieracium sp.	H	-	5
	Inula aspera	H	Med.-S.W. As.-Euras. step.	<2.5
	Jurinea blanda	H	Cauc. (Cauc. endemic)	18.5
	Leontodon asperrimus	H	Cauc.-S.W. As.	8
	Pterotheca sancta	Th	Med.-S.W. As.-Pon. (E. Med.-S.W. As.-Pon.)	47.5
	Scorzonera biebersteinii	H	Cauc.-S.W. As. (Cauc.-Ir.)	52.5
	Taraxacum praticola	H	Cauc. (Cauc. endemic)	2.5
	Tragopogon tuberosus	G	Cauc. (Cauc. endemic)	5
Boraginaceae	*Myosotis micrantha*	Th	Eur.-Med.-S.W. As.	16
Brassicaceae	*Alyssum tortuosum*	Th	Med.-Euras. step. (E. Med.-Euras. step.)	<2.5
	Arabidopsis thaliana	Th	Palaearctic	5
	Clypeola jonthlaspi	Th	Med.-S.W. As.-Tur.	<2.5
	Erysimum leptophyllum	H	Cauc. (S. Cauc. with Arm.-Kurd. irradiation)	<2.5
	Thlaspi perfoliatum	Th	Eur.-Anc. Med.	10.5
Campanulaceae	*Campanula hohenackeri*	Th	Cauc.-S.W. As.	2.5
Caryophyllaceae	*Cerastium glutinosum*	Th	Eur.-Med.	5
	Holosteum umbellatum	Th	Eur.-Anc. Med.	2.5
	Arenaria serpyllifolia	Th	Holarctic	<2.5
	Petrorhagia saxifraga	H	Med.-S.W. As.	5
Cistaceae	*Helianthemum salicifolium*	Th	Med.-S.W. As.	8
Crassulaceae	*Sedum caucasicum*	H	Cauc. (Cauc. with Arm.-Kurd.	2.5

			irradiation)	
Dipsacaceae	*Cephalaria media*	H	Cauc. (with E. Anat. irradiation)	<2.5
	Scabiosa columbaria	H	Eur.-Med.	18.5
Euphorbiaceae	*Euphorbia boissieriana*	H	Cauc. (Geo. endemic)	18.5
	Euphorbia iberica	H	Cauc.-S.W. As.	18.5
	Euphorbia seguieriana	H	Med.-Euras. step.	16
Fabaceae	*Astragalus bungeanus*	H	Cauc. (Cauc. endemic)	13
	Astragalus caucasicus	Ph	Cauc. (Cauc. with Arm.-Kurd. irradiation)	18.5
	Astragalus mollis	H	Cauc.-S.W. As.	2.5
	Medicago caerulea	H	Cauc.-Euras. step.	29
	Medicago minima	Th	Eur.-Anc. Med.	5
	Melilotus neapolitanus	Th	Med.-S.W. As.	60.5
	Onobrychis cyri	H	Cauc. (eastern Cauc. endemic)	47.5
	Trifolium campestre	Th	Eur.-Med.-S.W. As.	2.5
Hypericaceae	*Hypericum perforatum*	H	Med.-S.W. As.-Pon.	2.5
Lamiaceae	*Lamium amplexicaule*	Th	Palaearctic	5
	Scutellaria orientalis	Ch	Cauc. (with E. Anat. irradiation)	21
	Stachys atherocalyx	H	Med. (E. Med.)	21
	Teucrium nuchense	Ch	Cauc. (S. Cauc. endemic)	5
	Teucrium orintale	H	Cauc.-S.W. As.	<2.5
	Teucrium polium	Ch	Med.-S.W. As.-Pon.	37
	Thymus coriifolius	Ch	Cauc. (endemic of c. part of S. Cauc.)	21
	Ziziphora serpyllacea	Ch	Cauc. (with E. Anat. irradiation)	2.5
Malvaceae	*Alcea rugosa*	H	Cauc.-Euras. step.	2.5
Papaveraceae	*Papaver arenarium*	Th	Cauc.-S.W. As.	10.5
Polygonaceae	*Atraphaxis caucasica*	Ph	Cauc.-Mid. As.	<2.5
	Rumex tuberosus	G	Med.-S.W. As.	92
Ranunculaceae	*Thalictrum collinum*	H	Cauc.-Euras. step.	23.5
Rhamnaceae	*Paliurus spina-christi*	Ph	Med.-S.W. As.	2.5
Rosaceae	*Potentilla recta*	H	Palaearctic (W. Palaearctic)	40
Rubiaceae	*Asperula arvensis*	Th	Eur.-Med.	18.5
	Asperula glomerata	H	Cauc.-S.W. As. (E. Cauc.-S.W. As.)	2.5
	Galium album	H	Eur.-Cauc.	5
	Galium verum	H	Palaearctic	63
Santalaceae	*Thesium arvense*	H	Cauc.-Euras. step.	13
Scrophulariaceae	*Linaria simplex*	Th	Med.-S.W. As.	5
	Linaria genistifolia	H	Cauc.-Euras. step.	<2.5
Violaceae	*Viola kitaibeliana*	Th	Eur.-Med. (Mid. Eur.-Med.)	2.5
Monocotyledoneae				
Alliaceae	*Allium atroviolaceum*	G	Med.-S.W. As.-Euras. step. (E. Med.-S.W. As.-Pan.)	40
	Allium rotundum	G	Eur.-Med.	<2.5
	Allium rupestre	G	Cauc.-S.W. As. (with Crim. irradiation)	34
	Allium saxatile	G	Cauc.-Euras. step.	10.5
Cyperaceae	*Carex* schkuhrii	H	Cauc.-S.W. As.	29
Liliaceae	*Gagea chlorantha*	H	Cauc.-S.W. As.	21
	Gagea tenuifolia	H	Cauc.-S.W. As.	13
Poaceae	*Aegilops triuncialis*	Th	Med.-S.W. As.-Tur.	<2.5
	Anisantha sterilis	Th	Eur.-Med.-S.W. As.- Mid. As.	2.5
	Arrhenantherum elatius	H	Palaearctic	5
	Avena ludoviciana	Th	Anc. Med.	5

	Bothriochloa ischaemum	H	Anc. Med.	42
	Bromopsis biebersteinii	H	Cauc. (with E. Anat. & N. Ir. irradiation)	16
	Cleistogenes bulgarica	H	Cauc.-Euras. step. (Cauc.-Pan.-Pon.)	29
	Dactylis glomerata	H	Palaearctic	23.5
	Elytrigia repens	H	Palaearctic	8
	Festuca valesiaca	Th	Med.-S.W. As.-Euras. step.	34
	Koeleria cristata	H	Holarctic	81.5
	Lolium rigidum	H	Med.-S.W. As.	5
	Melica transsilvanica	H	Cauc.-Euras. step.	8
	Phleum phleoides	H	Palaearctic	58
	Poa pratensis	H	Holarctic	2.5
	Stipa pennata	H	Med.-Euras. step.	100

Abbreviations: Th - Therophyte (including biennial plants), H - Hemicryptophyte, G - Geophyte, Ch - Chamaephyte, Ph - Phanerophyte; Anat. - Anatolian, Anc. Med. - Ancient Mediterranean, Geo. - Georgian, Arm.-Kurd. - Armenia-Kurdistanian, Ir. - Iranian, As. - Asian, Cauc. - Caucasian, Crim. - Crimean, Eur. - European, Euras. step. - Eurasian steppe, Med. - Mediterranean, Pon. - Pontian, Pan. - Panonian, Tur. - Turanian; C. - Central, E. - East, Mid. - Middle, S. - South, S.W. - Southwest, W. - West.

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ბუნებრივი ვაციწვერიანი სტეპები (Stipeta pennatae) საქართველოს ეროვნული ბოტანიკური ბაღის (თბილისი) ტერიტორიაზე

ლაჩაშვილი ნ., ხაჩიძე მ., ერაძე ნ., ხეცურიანი ლ.

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(მიღებულია 20.10.2011)

რეზიუმე

შესწავლილია საქართველოს ეროვნულ ბოტანიკურ ბაღში (თბილისი) ველურად გავრცელებული ვაციწვერიანი თანასაზოგადოების (Stipetum pennatae) სტრუქტურა. მოცემულია გეობოტანიკურ-ფლორისტული დახასიათება. განხილული თანასაზოგადოება დიდი ხნის განმავლობაში არ იძოვება, რამაც თავისი ასახვა პოვა მის სტრუქტურაზე. კერძოდ, დიდი რაოდენობითაა დაგროვებული მკვდარი საფარი, რის გამოც ძალიან სუსტადაა განვითარებული ეფემერული სინუზია. შესაბამისად, ძლიერაა შემცირებული ერთწლოვან მცენარეთა ხვედრითი წილი და ცენოზური როლი, შემცირებულია ფლორისტული მრავალფეროვნება. ფლორისტული ანალიზის შედეგად დადგენილია, რომ შესწავლილი თანასაზოგადოება, რომლის შექმნაში მნიშვნელოვანია კავკასიური სახეობების როლი, ფლოროგენეზულ კავშირშია ერთი მხრივ, სამხრეთ-დასავლეთ აზიასთან და, მეორე მხრივ, ევრაზიის სტეპებთან. იკვეთება ხმელთაშუაზღვეთური ზეგავლენაც.

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EFFECT OF TEMPERATURE ON SEED GERMINATION OF *ARBUTUS ANDRACHNE* L.

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Abstract

Greek strawberry tree – *Arbutus andrachne* L. (Ericaceae) is species native to the Mediterranean region, The Middle East and south-western Asia. A few populations of *A. andrachne* naturally occur in coastal rocky limestones of Abkhazeti and Ajara. As a tertiary relict species *A. andrachne* is included in the Georgia Red List and needs to be protected. Small number of trees and weak natural regeneration of these populations indicate that seed germination and seedling establishment are insufficient. Seeds of *A. andrachne* possess a physiological dormancy that prevents synchronized and rapid germination.

The objective of this research was to assess germination ability of seeds subjected to different temperature regimes of incubation and to determine if seed dormancy is present in unharvested ripen fruits. Mean germination time (MGT), germination rate (GR), coefficient of variation of the germination (*CVt*), germination percentage (G), uncertainty (*U*) and synchrony (*Z*) were estimated for seeds incubated at 18° and 25°C. The seeds of *A. andrachne* show higher germination percentage G=56±3.16% at 18°C, as compared with G=21.72±4.32% at 25°C. The differences between other parameters where statistically insignificant ($p>0.05$): MGT=13.64±0.17 day, *CVt*= 22.69±2.16%, GR=0.07±0.01 day^{-1}, *U*=1.52±0.26 bit and *Z*=0.44±0.05 at 18°C compared to MGT=13.63±0.23 day, *CVt*=21.09±2.69 %, GR=0.07±0.03 day^{-1}, *U*=1.42±0.37 bit and *Z*=0.36±0.08 at 25°C. Our data show that the effect of temperature is stronger for the germination ability of seeds, whereas time, rate, coefficient of variation, uncertainty and synchronicity appear to be strictly genetically determined parameters less influenced by the temperature effects. Seeds from the unharvested ripen fruits revealed the ability to overcome dormancy. The MGT time took about 13 days and no pretreatment was necessary for seed germination. The results of present study indicate that seeds of *A. andrachne* possess non-deep type of physiological dormancy.

Key words: *Arbutus andrachne* L., seed germination parameters, nondeep physiological dormancy.

Introduction

Seed germination is a critical phase in the reproduction of higher plants as it is a starting point of entry of new organism into the ecosystem. Studies on seed germination are especially important in case of rare plants because they help to prepare an adequate conservation strategy for the endangered species.

Greek strawberry tree – *Arbutus andrachne* L. (Ericaceae) is a species native to the Mediterranean region, The Asia Minor and Northern Iraq. Evergreen trees and shrubs of *A.*

andrachne are widely cultivated as ornamental plants, since they don't grow in straight timbers, form convoluted branches and original bright terracotta bark that peels every year. In addition to its use as fresh fruit, species is a potentially important source of antioxidants [Serçe et al., 2010]. A few populations of *A. andrachne* naturally occur in coastal rocky limestones of Abkhazeti and Ajara [Flora of Georgia, 1980]. Small number of trees and weak natural regeneration of these populations indicate that seed germination and seedling establishment are insufficient. As rare relict plant *A. andrachne* is included in the Georgia Red List and needs to be protected. Seeds of *A. andrachne* are known to be physiologically dormant [Kose, 1998; Tilki, Guner, 2007]. It means that fully developed embryo is characterized by retarded growth due to endogenous inhibitors that block germination [Finch-Savage, Lubner-Metzger, 2006]. However, the type of physiological dormancy is not established.

The aim of this study is to assess germination ability of seeds subjected to different temperature regimes of incubation. Our main question is: do the seeds of *A. andrachne* from the unharvested ripen fruits have the ability to overcome dormancy. The second task is to determine the type of physiological dormancy of seeds.

Materials and Methods

Ripe fruits of *A. andrachne* were collected in Georgian National Botanical Garden from the mature tree growing at the plot of Rare and Medicinal Plants of Caucasus in January, 2011. After 24 hours of soaking, pulp was removed by maceration in sieve with mesh size ca. 1.5 mm. Seeds that sink when soaked in water were used in the germination experiments. 30 seeds per dish were placed in Petri dishes, with filter paper moistened with distilled water. The treatments were arranged in completely randomized design with 3 replicates. Germinated seeds were counted every 4 days for 3 weeks. Seed germination was defined as the appearance of a radicle, at least 2 mm long. Measurements and statistics of the germination process followed [Ranal, Santana, 2006]. Mean germination time was calculated by the expression

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i},$$

where t is a time from the start of the experiment to the i^{th} observation day, n_i – number of seeds germinated in the ith time and k - last time of germination. Coefficient of variation of the germination time was calculated by the expression

$$CV_t = \frac{s_t}{\bar{t}} 100,$$

where S_t is a standard deviation of the germination time and t - mean germination time. Mean germination rate (GR) was calculated as a reciprocal of the mean germination time. Uncertainty of the germination process was calculated by the expression

$$U = -\sum_{i=1}^{k} f_i \log_2 f_i, \text{ being } f_i = \frac{n_i}{\sum_{i=1}^{k} n_i},$$

where n_i is a number of seeds germinated on the i^{th} time, and k – last day of observation. Synchrony of germination Z was calculated by the expression

$$Z = \frac{\sum_{i=1}^{k} C_{n_i,2}}{C_{\sum n_i,2}},$$

Being $Cn_i,2=n_i(n_i-1)/2$ where $Cn_i,2$ is a combination of the seeds germinated in the ith time, two by two, and n_i is a number of seeds germinated in the i^{th} time. Results of germination experiments are expressed in percentage (±S.D.).

Results and Discussion

Ripe unharvested fruits dry up staying attached to the tree for months. The number of seeds per fruit varies from 2 to 17, with an average 12. Mature seed is up to 2.5 mm long, contains a colourless to cream well-developed embryo which occupies most of the seed length (Fig. 1, a).

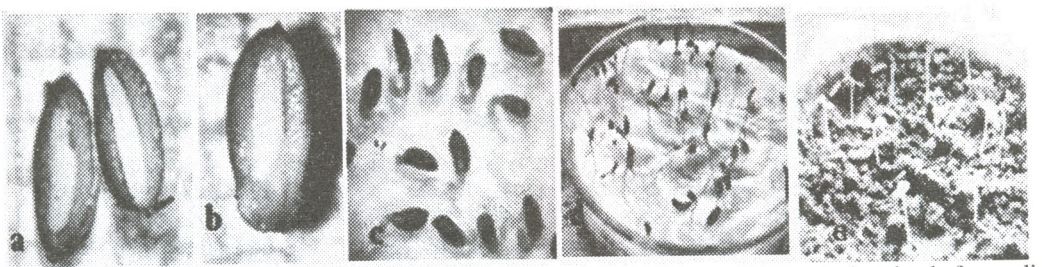

Fig. 1. Longitudinal sections of seed of *A. andrachne*; a - before the incubation, b - before radicle protrusion X 15, c, d - germinated seeds, e - seedlings.

Study was conducted to determinate if big embryos (i.e. high embryo length / seed length) in mature seeds are underdeveloped. We should therefore have expected that they would grow inside the seed prior to germination, and seeds would require 2-4 month of cold stratification to overcome dormancy. However, our experiments indicate that the opposite events take place. Thus, embryo length increases insignificantly ($F=1.4$, $p< 0.05$) at the moment of radicle protrusion. Moreover, fully developed embryos (i.e. cotyledons, embryonic axis, and radicle) are clearly distinguishable in longitudinal sections (Fig. 1, b).

Germination ability of seeds incubated at 18°C is 56.00±3.16 %, which is 35% higher than at 25°C (Fig. 2).

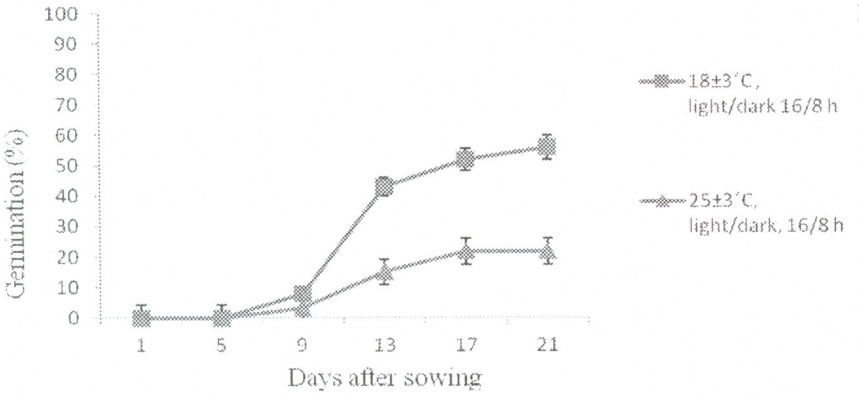

Fig. 2. Relationship between temperature of incubation and seed germination ability of *A. andrachne*. Mean of three replicates ±S.D. are presented.

Germination declined with an increasing temperature. The values of measured germination parameters are presented in the Table 1.

Table 1. Germination measurements (mean±SD) of *A. andrachne* seeds collected in the National Botanical Garden, Tbilisi in January, 2011. *G* - germinability, MGT - mean germination time, CV_t - coefficient of variation of the germination time, GR - mean germination rate, *U* - uncertainty, *Z* - synchrony.

Matrix	*G* (%)	MGT (day)	*CVt* (%)	GR (day-1)	*U* (bit)	*Z*
1. 18° C	56.00±3.16	13.64±0.17	22.69±2.16	0.07±0.01	1.52±0.26	0.44±0.05
2. 25° C	21.72±4.32	13.63±0.23	21.09±2.69	0.07±0.03	1.42±0.37	0.36±0.08

Our study revealed that germination ability of seeds incubated at 18°C was markedly higher (56% compare with 21.72% at 25°C). These data indicate that temperature has a significant ($p<0.05$) effect on seed germinability in *A. andrachne*. At the same time, the influence of temperature does not seem sufficient to the time spend to germinate. Thus, MGT values were almost identical for both temperature regimes: 13.64±0.17 at 18° and 13.63±0.23 at 25°C. It must be emphasized that the seeds from the unharvested ripen fruits have an ability to overcome dormancy naturally and germinate as undormant ones. As MGT is used to evaluate time spent to emerge, we conclude that seeds of *A.andrachne* possess a non-deep physiological type of dormancy according to modern classification of seed dormancy [Smith et al., 2003; Baskin, Baskin, 2004].

Our data showing no comparable differences in measured parameters of *CV,* GR, *U, Z* may be interpreted as an evidence that the variability as well as the germination rate, degree of spreading of germination through time and germination overlapping are genetically encoded constant parameters less influenced by abiotic factors. In other words, if seeds are able to germinate the measured values are expected to be similar among treatment regimes.

Seed germination starts with the uptake of water by dry seed and terminates with the visible penetration of the structures surrounding the embryo by the embryonic axes [Bewley, 1997]. This process is governed by two major antagonistic phytohormones, abscisic acid (ABA), which inhibits seed germination and post-germination growth and gibberellin (GA), which promotes mentioned processes [Gulber et al., 2005]. The breakdown of seed dormancy is influenced by both environmental and intrinsic signals [Koornneef et al., 2002] which are also mediated by these two counteracting phytohomones. Results of our study revealed that physiologically dormant seeds of

A. andrachne from unharvesed ripen fruits demonstrate an ability to germinate with MGT about 13 days. We suggest that this fact can be explained by the effect of low winter temperature which shifts the hormonal balance toward the activation of gibberellins synthesis providing developmental transition from seed dormancy to germination.

Summarizing the data obtained we can conclude that effect of temperature on seed germinability of *A. andrachne* is stronger than the genetic component which strictly determines the species specific properties: the time spent to germinate, speed, uncertainty and synchrony. The upper limitation of incubation temperature required for the maximum germination percentage is under 25°C. MGT 13 days reflects a non-dormant condition of seeds. These observations provide evidence that seeds of *A. andrachne* have non-deep type of physiological dormancy, which naturally overcomes during winter in ripen unharvested fruits.

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ტემპერატურის გავლენა *Arbutus andrachne* L. თესლის გაღივებაზე

შაქარიშვილი ნ., ასიეშვილი ლ., ერაძე ნ., სირაძე მ.

ილიას სახელმწიფო უნივერსიტეტის ბოტანიკის ინსტიტუტი

(მიღებულია 11.08.2011)

რეზიუმე

ხემარწყვა (Ericaceae) საქართველოს წითელ ნუსხაში შეტანილი რელიქტური, იშვიათი, მაღალდეკორატიული, თაფლოვანი მცენარეა, რომლის არეალი ხმელთაშუაზღვეთს, მცირე აზიასა და ჩრდილოეთ ერაყს მოიცავს. საქართველოში მისი მცირერიცხოვანი პოპულაციები მხოლოდ აფხაზეთისა და აჭარის კლდოვან კირქვიანებზე გვხდება. სახეობის სუსტი ბუნებრივი განახლება თესლის გაღივების პრობლემებს უკავშირდება. *A. andrachne*-ს თესლს ფიზიოლოგიური მოსვენების პერიოდი ახასიათებს, რაც სწრაფ და სინქრონულ გაღივებას აბრკოლებს.

კვლევის მიზანს *A. andrachne*-ს თესლის გაღივებაზე ტემპერატურის გავლენის შესწავლა და ფიზიოლოგიური მოსვენების ტიპის დადგენა წარმოადგენდა. გაღივების ძირითადი პარამეტრები: საშუალო დრო (MGT), სიჩქარე (GR), ვარიაბელობა (CV_t) პროცენტულობა (G), განუზღვრელობა (U) და სინქრონულობა (Z) ინკუბაციის ორი ტემპერატურული რეჟიმის (18° და 25°C) პირობებისათვის განისაზღვრა. ტემპერატურა მნიშვნელოვან ($p<0.05$) გავლენას მხოლოდ თესლის გაღივების უნარზე ახდენს: 18°C-ზე G=56±3.16 %, რაც 35%-ით აღემატება 25°C-ზე გაღივებული თესლების რაოდენობას. ინკუბაციის განსხვავებული რეჟიმის პირობებში გაღივების უნარის მქონე თესლების გაღივების დანარჩენი პარამეტრები მსგავსია და ტემპერატურის ცვლილებაზე ნაკლებადაა ($p>0.05$) დამოკიდებული: MGT=13.64±0.17 დღეს, CVt= 22.69±2.16%, GR=0.07±0.01 დღე$^{-1}$, U=1.52±0.26 ბიტს და U =0.44±0.05 (18°C-ზე). 25° C-ზე გაღივებისას MGT=13.63±0.23 დღეს, CVt=21.09±2.69%, GR=0.07±0.03 დღე$^{-1}$, U=1.42±0.37 ბიტს და Z=0.36±0.08. მიღებული შედეგები შესწავლილი ნიშნების გენეტიკურ განსაზღრულობაზე მიუთითებს. თესლის გაღივების საშუალო დრო 13 დღეა - ვადა, რომელიც მოსვენების პერიოდის მქონე თესლს არ ახასიათებს. ამ მონაცემის საფუძველზე დადგენილია *A. andrachne*-ს თესლის არაღრმა ფიზიოლოგიური მოსვენების ტიპი, რომლის ბუნებრივი შეწყვეტა ზამთრის პერიოდში ხდება.

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THE ABUNDANCE AND DIVERSITY OF *VIBRIO* SPECIES NOT PATHOGENIC TO HUMANS IN GEORGIAN AQUATIC ENVIRONMENT

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Abstract

The diversity, quantitative abundance and seasonal distribution of *Vibrio* species not pathogenic to humans in the Georgian aquatic environment have been studied. The samples were collected in 2006-2009 in the Black Sea coastal zone and freshwater reservoirs nearby Tbilisi. The isolated presumptive *Vibrio spp.* were identified based on biochemical profiles. Non-clinical *Vibrio spp.* isolates were divided into 3 similarity groups according to their salt requirements. Seven non-pathogenic *Vibrio* species were identified: *V. orientalis, V. marinus, V. natriegens, V. pelagius, V. campbellii, V. splendidus* and *V. nereis.*

Key words: Georgia, water reservoirs, vibrios, diversity, seasonality.

Introduction

Members of the *Vibrionaceae* family are ubiquitous in the marine environment and have been found in coastal and open marine environments, surface and deep waters, as free-living populations and in association with marine animals, algae and detritus. The vibrios are motile, gram-negative, curved to comma-shaped bacteria. A number of vibrios have growth optima at brackish salinities and are routinely detected in coastal estuaries, while others are also found in association with freshwater systems [Farmer and Hickman-Brenner, 2001; Heidelberg et al., 2002; Thompson et al., 2003]. The number of reported species in the genus *Vibrio* increased rapidly in the last decade and presently comprises 65 environmental species [Thomspon et al., 2006]. Twelve of them (*V. cholerae, V. parahaemolyticus, V. vulnificus, V. mimicus, V. metchnikovii* etc.) are of clinical importance since they have been associated with skin infections and severe gastrointestinal disorders in humans [Joseph et al., 1982; Andrews, 2004; Pérez-Rosas and Hazen, 1998; Lesmana M, 2002]. Majority of *Vibrio* species have been attributed to group of bacteria not pathogenic to humans [Thompson et al., 2006; Noriega-Orozco et al., 2007]. The ecology of clinically important *Vibrio* species, including *V. cholerae* and *V. parahaemolyticus,* in the Georgian water environment have been recently studied [Grim et al, 2009; Whitehouse et al.,

2010; Janelidze et al., 2010] while there are almost no data on abundance and species composition of non pathogenic *Vibrios* in the geographic area.

The aim of the present study was the assessment of abundance, distribution and diversity of non-pathogenic *Vibrio spp.* in water reservoirs in Georgia. In our previous studies 11 clinically important *Vibrio species* were registered. In this paper we analyze the other larger group of *Vibrio spp.* isolates which were not attributed to the group of human pathogenic *Vibrios* and were regarded as *Vibrio spp.* presumptively non-pathogenic to humans.

Materials and Methods

Regular monthly monitoring has been carried out in 2006-2009 in the Black Sea coastal zone of Georgia and in the freshwater reservoirs in Tbilisi surroundings: Lisi Lake, Tbilisi Sea, Kumisi Lake. Periodically samples were taken from Nuri Lake (Batumi, Georgia).

Isolation and culturing of bacteria from environmental sources was done according to standard protocols. Collected isolates have been stored at -80^{0}C in 25% glycerol.

Bacterial isolates were studied by conventional bacteriological methods [Huq et al., 2006; Grim et al., 2010). Biochemical characterization of strains was done according to 13 different criteria including salt requirements, growth on specialized media, utilization of amino acids and carbohydrates. For comparison purposes identity of single representatives of similarity groups by phenotypic properties of isolates was confirmed by using API 20E and API 20NE Tests (Biomereux, France).

Biochemical identification of non-pathogenic *Vibrio* species was done using the algorithm designed for presumptive *Vibrio spp.* isolates, based on thirteen parameters mentioned above. If the *Vibrio* isolates' properties coincided with those of the reference strains of 12 clinically important vibrios, a percentage of affinity was calculated using different weighted factors for various biochemical parameters (P, [P]).

Results and Discussion

During the 30 months monitoring we collected in total 2043 presumptive *Vibrio* isolates and characterized them by protocol of phenotypical profiling with corresponding algorithm. 1150 isolates were identified as human pathogenic *Vibrios*, majority represented by *V. cholerae, V. parahaemolyticus, V. vulnificus, V. metchnikovii, etc.* [Grim et al., 2010; Whitehouse et al., 2010; Tediashvili et al., unpublished data]. The rest - 893 of *Vibrio* isolates comprised a large group of vibrios of non-pathogenic to humans since the standard biochemical identification used in all previous identification protocols did not detect 12 clinical *Vibrio* species within this group. For broadening the spectrum of identifiable environmental *Vibrio* species we decided to modify the previous Algorithm (based on the map of markers, 13 parameters) to make it more distinctive by introducing the marker's weight and threshold values. The application of the new modified algorithm to the same group of 893 *Vibrio* isolates resulted in additional identification of 221 isolates on species level. Among them 168 were added to already existing group of clinically important *Vibrios*: 35 have been attributed to *V. cholerae* and 43 - to *V. mimicus*, 47 - *V. parahaemolyticus*, 27 - *V. vulnificus*, 12 - *V. metschnikovii* and 4 - *V. alginolyticus.* Besides, 4 isolates were characterized as *Aeromonas spp.* and 4 - as *Proteus spp.*

53 *Vibrio spp.* isolates identified at species level, appeared to be new to this study and represent the group of vibrios non-pathogenic to humans. In total 7 non-clinical *Vibrio* species were revealed in Georgian aquatic environment: *V. orientalis, V. marinus, V. natriegens, V. pelagius, V. campbellii, V. splendidus* and *V. nereis.*

The rest 661 isolates remain unidentified by biochemical profile and were regarded as non-pathogenic *Vibrio spp.* More information on species composition in this group of isolates requires further molecular identification by 16S rRNA sequencing. At this stage we divided them into similarity groups, based on their biochemical characteristics, mostly salt requirements.

661 isolates of suspected *Vibrios spp.* and 53 identified non-pathogenic *Vibrio spp.* were divided into 3 similarity groups (Fig. 1). The first group - non halophilic *Vibrios* (237 isolates). The second – intermediate group (270 isolates) and the third group - halophilic *Vibrios* (207 isolates).

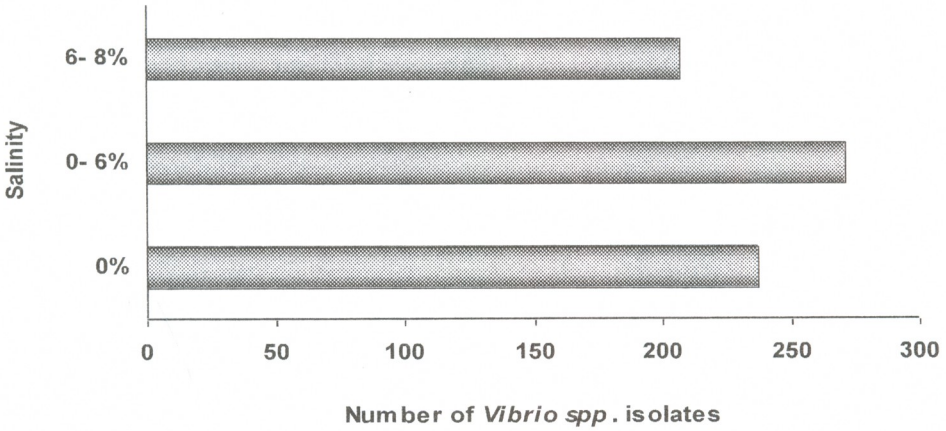

Fig. 1. The grouping of non-clinical *Vibrio spp.* based on salt requirements.

The preliminary assessment of diversity and quantitative abundance of non-pathogenic *Vibrio spp.* (53 isolates) and not identified to species level *Vibrio spp.* (661 isolates) in Georgian aquatic environment in different seasons was done including the Black Sea sites: Supsa, Chorokhi, Boulevard and Green Cape, also freshwater reservoirs nearby Tbilisi: Lisi Lake, Kumisi Lake and Tbilisi Sea.

The analysis of isolation time and site of collected non-clinical *Vibrio spp.* revealed their seasonal distribution (Fig. 2). The warm season - summer and autumn months appear to provide favorable conditions for *Vibrio spp.* propagation in water environment of Georgia.

The majority of non-pathogenic *Vibrio spp.* isolates (455 isolates) were found in freshwater reservoirs, and 206 – in the Black Sea coastal zone. Marine isolates of non-pathogenic *Vibrio spp.* were mainly (72 isolates) collected from Supsa water samples followed by samples from Chorokhi (58), Batumi Boulevard (41) and Green Cape (35) correspondingly.

In general, the Black Sea water samples were characterized by less *Vibrio spp.* diversity (Fig. 3). 5 *Vibrio* species were found among 206 of non-pathogenic *Vibrio* isolates: *V. marinus, V. natriegens, V. splendidus* were isolated from Green Cape water samples, *V. orientalis, V. natriegens, V. pelagius, V. splendidus* from Chorokhi water samples and *V. orientalis, V. natriegens, V. Pelagius* - from Supsa water samples. Only one identified species - *V. natriegens* was revealed in the Boulevard water samples.

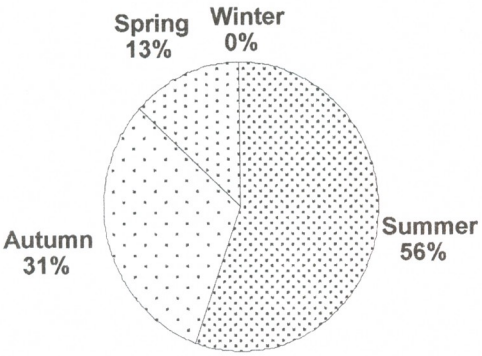

Fig. 2. Distribution of Georgian isolates of non-pathogenic *Vibrio spp.* isolates by seasons

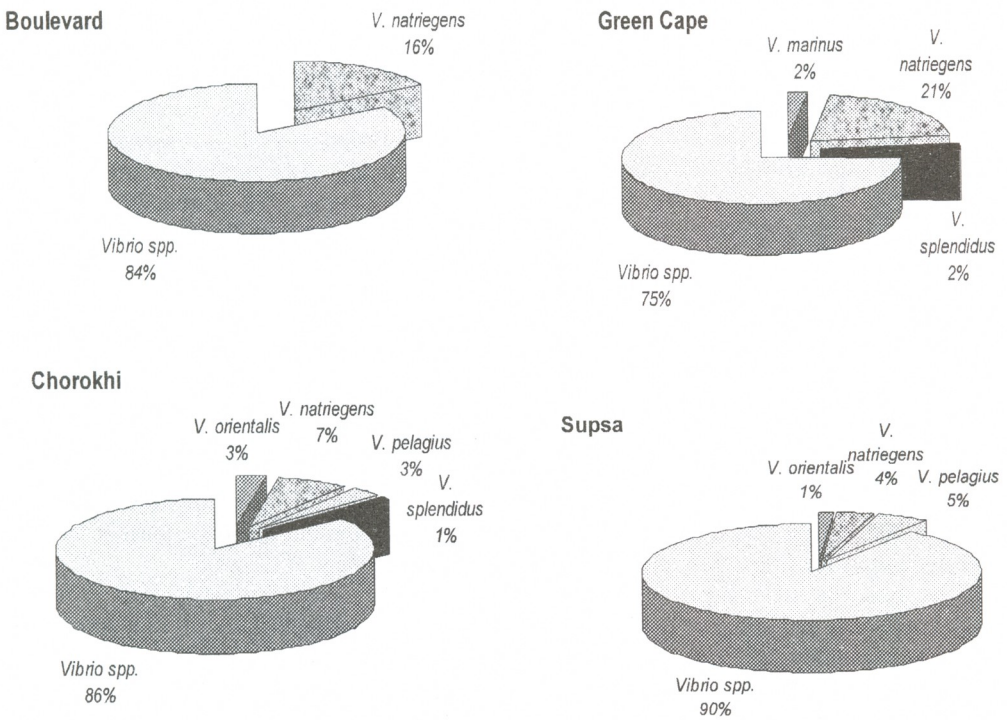

Fig. 3. The biodiversity of non-pathogenic *Vibrio spp.* in the Black Sea coastal zone of Georgia.

It should be noted that *V. natriegens* was also detected in the studies on direct detection of *Vibrios* done by PCR- Electrospray Ionization – Mass Spectrometry (PCR/ESI-MS) method [Whitehouse et al., 2010]. The DNA of this bacterium was found in the water samples from all Black Sea sites (sites: Chorokhi, Supsa, Boulevard and Green Cape) and also in freshwater reservoirs: Kumisi Lake, Lisi Lake and Tbilisi Sea. Although *V. natriegens* has not been found among freshwater isolates.

The Georgian freshwater reservoirs appeared to differ from marine sites by the diversity of non- pathogenic *Vibrio spp.* isolates (Fig. 4). In total six non-clinical species have been identified in the studied inland reservoirs. Among them *V. orientalis*, *V. marinus*, *V. pelagius*, *V. campbellii*, *V. splendidus*, *V. nereis*. *V. splendidus* were detected in all three reservoirs near Tbilisi, while *V. campbelii* was found in Lisi lake and *V. orientalis* - only in Nuri Lake. It should be noted that *V. campbellii* and *V. nereis* have been detected in the freshwater reservoirs only, while *V. orientalis, V. marinus, V. pelagius, V. splendidus* were isolated from both - marine and freshwater samples.

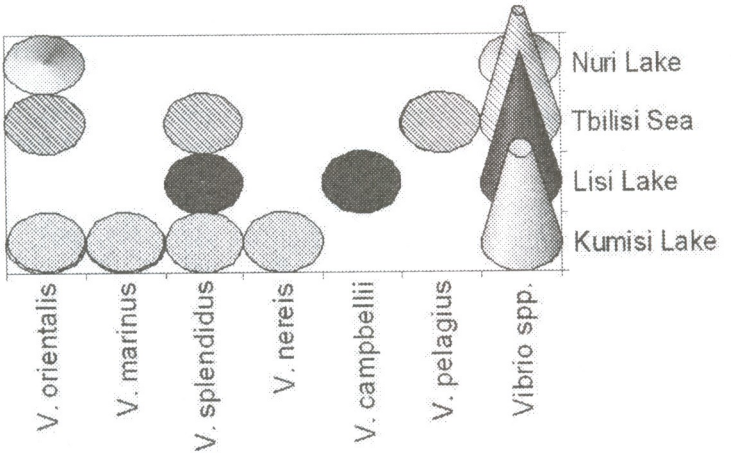

Fig . 4. The distribution of non –clinical *Vibrio spp.* in some inland water reservoirs of Georgia

Although above discussed *Vibrio* species are not pathogenic to humans, *V. campbellii* , *V. ordalii, V. splendidus* and *V. pelagius* can cause disease (vibriosis) with various manifestations in several fish species and shrimp [Wilk et al., 1995; Thompson et al., 2006; Noriega-Orozco et al., 2007].

Conclusion

Various biochemical tests were used to identify 661 non-pathogenic *Vibrio spp* isolates, collected in Georgian aquatic environment, 206 among them - from the Black Sea coastal zone and 455 – from the inland reservoirs. Seven species of various non-pathogenic *Vibrios* were revealed: *V. orientalis, V. marinus, V. natriegens, V. pelagius, V. campbellii, V. splendidus and V. nereis.*

Six out of seven total identified non-clinical species were found in freshwater samples and 5 - in marine water. *V. orientalis* quantitatively predominated in the studied freshwater samples, while *V. natriegens* was prevalent in the Black Sea water samples.

Environmental non-pathogenic *Vibrio spp.* isolates were divided into similarity groups based on their biochemical characteristics, their seasonal distribution and abundance in monitored water bodies was assessed as well.

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ადამიანისათვის არაპათოგენური ვიბრიონების გავრცელება და მრავალფეროვნება საქართველოს წყლიან გარემოში

მითაიშვილი ნ.[1,2], ცხვედიანი ა.[1,2], ელბაქიძე თ.[1,2], ქოქაშვილი თ.[1], ნატროშვილი გ.[1], ქაჯაია გ.[2], თედიაშვილი მ.[1].

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(მიღებულია 24.10.2011)

რეზიუმე

წარმოდგენილი კვლევის მიზანი იყო ადამიანებისთვის არაპათოგენური ვიბრიონების გავრცელების და მრავალფეროვნების შესწავლა საქართველოს წყლიან გარემოში. წყლის სინჯები აღებული იყო 2006-2009 წლებში საქართველოს შავი ზღვის სანაპირო ზოლსა და თბილისის მიდამოებში არსებულ წყალსაცავებში: ლისისა და კუმისის ტბაში, და თბილისის ზღვაში. შესწავლილ იქნა 893 არა-კლინიკური *Vibrio spp.*-ის იზოლატი, რომლებიც მარილისადმი დამოკიდებულების მიხედვით დაიყო 3 ჯგუფად. მათგან 53 იზოლატი მიეკუთვნა *Vibrio*-ს 7 სახეობას: *V. orientalis, V. marinus, V. natriegens, V. pelagius, V. campbellii, V. splendidus* და *V. nereis.*

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Entomology

USE OF ENCYRTID (HYMENOPTERA: CHALCIDOIDEA, ENCYRTIDAE) FAUNA TO ESTIMATE LIKE NUMBER OF SCALE (HEMIPTERA: COCCOIDEA) FAUNA IN GOLCUK NATURAL PARK, TURKEY

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Abstract

Encyrtid species collected by malaise trapping in Golcuk Natural Park, Turkey, together with previous records of encyrtids from the surrounding regions (Isparta Province), were used to estimate the number of scale species likely present in the Park. From these data we found 51 species of encyrtids known to be parasitoids of scales in Isparta province. These parasitoids are associated with in total 168 scale species as hosts worldwide; however, only 60 of those scales have been recorded from Turkey. The use of Chi-square statistics predicts that the list of scales in Turkey is very incomplete and the probability of finding more scale species in the area is very high, which is proved by various parasitoids recorded here.

Key Words: Golcuk, Encyrtidae, Coccoidea, Turkey

Introduction

Many phytophagous insect species seem, on average, to be considerably less abundant than ought to be possible based on the availability of their resources and, indeed, it is often difficult to record them in particular study areas because of their low densities. These low densities, especially for scale, can be due to the action of specialized natural enemies (especially parasitoids). Information on parasitoid community structure is important for several reasons. First, shared parasitism may be a significant factor structuring the whole insect community [Memmott et al., 1994]. Parasitoids may, for example, determine the number of herbivores that can coexist in a common habitat [Lawton, 1986; Holt & Lawton, 1993]. While host-parasitoid population dynamics and abundance have often been studied [Krivan, 1997; Maron & Harrison, 1997], no publications have previously attempted to use parasitoid fauna in a region to forecast the species assemblage used by them as hosts. The goal of our study is to make preliminary estimates of scale species likely to be present in the study area based on records of the local scale-attacking parasitoid fauna.

Materials and Methods

Insect samples were collected from March to October, 2009, using the malaise traps located in the Golcuk Natural Park (GNP). Malaise traps were placed in two locations, first at Pilav Tepe (altitude 1520 m) and second in an area that has been reforested with Acacia and which is near the main entrance of GNP (altitude 1414 m). The malaise traps were checked and material was collected every 10 days. After collecting the captured insects, they were sorted according to the orders, then Hymenotperans were sorted according to the superfamilies and families. Later Chalcidoidea superfamily species were Critical Point Dried and the Encyrtidae material was card mounted and slide mounted according Noyes (2010). Encyrtidae specimens were sorted to genera and, thereafter, to species. Host records for encrytid species found in samples were taken from Noyes (2010) and Ben-Dov et al. (2010). For parasitoids from samples that could be determined up to the genus, we used only those host records that themselves were listed for parasitoids in the given genus for which parasitoid species was not given. This was done not to lose track of any potential scale that might be located in the study area and only for encrytid genera known to be scale parasitoids. All voucher specimens are deposited in the collection of Entomology and Biocontrol Research Centre, Agrarian University of Georgia, Tbilisi, Georgia.

Results and Discussion

To test the possibility that more scale species would exist in the study area, we used Chi-Square analysis [Moore & McCabe, 1998].

We used the hypothesis test given below.

H_0: There is no significant difference between Turkey and World according to proportion of species in family groups.

H_a: There is significant difference between Turkey and World according to proportion of species in family groups.

The null hypothesis will be rejected, when we will find the chi-square statistics is greater than the table value of chi-square (or for small p-value) at a particular significance level.

Fifty one species of encrytids were recorded from recent or previous studies in the study region [Japoshvili & Karaca, 2009; Japoshvili & Celik, 2010; Japoshvili, 2011]. At least 168 scale species worldwide are known to be attacked by this group of 51 encrytids. However, only 60 of these 168 scales have been recorded from Turkey [Noyes, 2010; Ben-Dov et al. 2010] (Table 1). List of recorded species of encyrtids during survey is following: *Anagyrus alienus, A. aligarhensis, A. descriptus, A. schmuttereri, A. securicornis, Aphycus moravicus, Aschitus golcukus, Baeocharis* sp., *Blastothrix gurselae, Cerapterocerus mirabilis, Charitopus bulentyasari, Ch. fulviventris, Ch. ismailkaracai, Cheiloneurus claviger, Ch. elegans, Ch. paralia, Coccidencyrtus* sp., *Discodes coccophagus, Dusmetia ceballosi, Encyrtus infidus, Ericydnus apterogenes, E. karakalensis, E. robustior, E. sipylus, E.* sp.dif. *aeneus, E.* sp. dif. *luka* and *pheliococci, E. nino, E.* sp. dif. *nino, E.* sp. dif. *sipylus, Leptomastix dactylopii, L. ephyra, Mahencyrtus comara, Mayrencyrtus discolor, M. imandes, Mayridia procera, Metaphycus lounsburyi, M. petitus, M. stagnarum, M. stanleyi, M. swirskii, M. zebratus, Microterys bellae, M. cneus, M. darevskii, M. hortulanus, M. sylvius, Pseudococcobius obenbergeri, Rhopus flavidus, Subprionomitus* sp., *Trichomasthus* sp., *Zaomma lambinus.*

We excluded four groups of family (aclerdidae, asterolecanidae, eriococcidae, kermesidae), since those groups have less than five expected count, so we based our calculations on Coccidae, Diaspididae, and Pseudococcidae families. From the Table 1. the chi-square statistics is found as 4.984 having 0.083 p-value which is less than 0.1 significance level. Therefore, the null hypothesis can be rejected. So, it can be concluded that there is significant difference between

Turkey and World according to proportion of species in family groups. That means that there is still more species that can be found in Turkey.

Table 1. Number of scale species, by world or by Turkey, associated as hosts with parasitoid species recorded in Isparta province, Turkey.

Family	Turkey	World
1. Aclerdidae	-	1
2. Asterolecanidae	3	4
3. Coccidae	22	73
4. Diaspididae	17	27
5. Eriococcidae	3	8
6. Kermesidae	1	2
7. Pseudococcidae	14	53
Total	60	168

Therefore, given that 141 species of encyrtids are known in Turkey [Japoshvili & Noyes, 2005; Japoshvili & Karaca, 2007; Japoshvili et al., 2009; Japoshvili & Celik, 2010; Kaydan & Japoshvili, 2010; Japoshvili, 2011], the number of scales known from Turkey will potentially, at least, double as the fauna should be subjected to more intensive collecting.

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ენცირტიდების (Hymenoptera: Chalcidoidea, Encyrtidae) ფაუნა, როგორც გოლჯუქის ბუნებრივ პარკში ფარიანების (Hemiptera: Coccoidea) ფაუნის შესაძლო რაოდენობის დადგენის საშუალება

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რეზიუმე

გაანალიზებულია გოლჯუქის ბუნებრივ პარკსა და ისპარტის მოსაზღვრე რეგიონების მალაის ხაფანგით შეგროვებული ენცირტიდები, რათა დაგვედგინა პარკში არსებული ფარიანების შესაძლო რაოდენობა. მიღებულმა შედეგებმა აჩვენა, რომ ისპარტის პროვინციაში აღრიცხულია 51 სახეობის ენცირტიდა. აღრიცხული პარაზიტოიდები დაკავშირებული არიან 168 სახეობის ფარიანასთან მსოფლიოში, თუმცა თურქეთში მათთვის ცნობილია მხოლოდ 60 ფარიანა მასპინძელი. Chi-square სტატისტიკურმა ანალიზმა აჩვენა, რომ თურქეთის ფარიანების ფაუნა არაა სრულად შესწავლილი და დიდი ალბათობაა, რომ აღრიცხული იყოს მეტი ფარიანა, რასაც ამ ტერიტორიაზე აღრიცხული პარაზიტოიდების ფაუნაც აჩვენებს.

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A PRELIMINARY STUDY OF THE CARABID DIVERSITY AND COMPOSITION IN BORJOMI-KHARAGAULI NATIONAL PARK, GEORGIA

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Abstract

Carabidae diversity was investigated at Borjom-Kharagauli National Park (BKNP), Georgia. The study area was divided into five sampling sites with different plant associations representing all main habitat types of BKNP: The mesophilic valley in Baniskhevi; Kvabiskhevi, in this dry valley habitat the influence of the western humid climate is minor; Likani 1, coniferous forest; Likani 2, mixed forest and Bairagebis seri, subalpine meadow.

Twenty seven species were recorded during the survey. Carabids were most abundant at a site Bairagebis seri, featuring with high diversity of herbs. Forty seven percent of all sampled individuals were found there. The study revealed that this site provided special micro-habitats for Carabidae fauna. Nineteen species were recorded only at one site. *Carabus armeniacus, C. ibericus, C. puschkini, C. septemcarinatus* and *Cychrus aeneus* were identified as indicator species. The abundance of carabid species differed significantly among some studied habitats.

Key words: Carabidae, biodiversity, Borjomi-Kharagauli, Georgia

Introduction

In recent decades, humans have more than ever been changing the world's ecosystems to meet the growing demands for food, freshwater, timber, fiber, fuel and minerals [Anonymous, 2005]. Biodiversity in itself provides a range of services, including aesthetic, cultural and recreational values, as well as goods that have direct use value, and also enhances many other ecosystem services on which humans depend [Gaston & Spiser, 1998; Bulte et al., 2005]. There is a large body of research suggesting that natural ecosystem properties greatly depend on biodiversity and that the functioning of ecosystems is associated with biodiversity [Mertz et al., 2007]. Biodiversity is also infiltrating administrative language, particularly after the UN global Conference on the Environment and Development held in 1992 [UNEP, 1992; Haila & Kouki, 1994]. The conference declared preservation of biodiversity as one of the major elements of sustainable development [Zilihona & Nummelin, 2001].

The history of protected territories in Eurasian country, Georgia dates back to the Middle Ages when the territories were used by local feudal lords for hunting. In 1862, the brother of the

ruling Russian Emperor Mikhail Romanoff was appointed Viceroy of the Russian Empire to Transcaucasia. He was greatly impressed by the beauty of the Borjomi Gorge and decided to build a summer residence there. Soon after, he fenced a large part of the forest and forbade the felling of trees and hunting without permission. After more than a century of social and political turbulence the founders of the Borjomi-Kharagauli used the territory established by Mikhail Romanoff as the basis for the first national park in the Caucasus. In 1995, Borjomi-Kharagauli National Park was created with the support of the World Wide Fund for Nature (WWF) and the German government, and was officially inaugurated in 2001 [BKNP, 2006]. BKNP is currently listed by the IUCN in its second category of conservation significance.

The Caucasus region is on the list of 34 hot spots for wildlife globally. High biodiversity and endemism are characteristic of the region [Anonymous, 2007]. The number of plant and animal species per unit of land area exceeds the average global index by a factor of 100 and the diversity of invertebrate fauna, including insects, still requires further investigation [Abuladze et al., 2001]. In 2005, the government of Georgia instituted the 'National Biodiversity Strategy and Action Plan – Georgia'. In the section 'Species and habitats', the development of an inventory of fauna was specified as one of the priority areas.

The Borjomi district is located on the border between the Caucasus and Irano-Anatolian hotspots and this is reflected in the flora of the BKNP. However, the vegetation of this national park has not been studied in detail. In the study area, which was in the southern part of the National Park, the vegetation is different due to the geographical proximity of eastern Georgia and large masses of humid air entering via the Ajara-Imereti mountain range [Abuladze et al., 2001]. The characteristic plants include Imeretian buckthorn (*Rhamnus imeretina*), yew (*Taxus baccata*), Pontic and Caucasian rhododendron (*Rhododendron ponticum* and *Rh. caucasicum*), cherry laurel (*Laurocerasus officinalis*), holly (*Ilex colchica*), Colchian ivy (*Hedera colchica*), sweet chestnut (*Castanea sativa*), beech (*Fagus orientalis*), hornbeam (*Carpinus caucasica*), linden (*Tilia caucasica*) and Cochian oak (*Quercus hartwissiana*), among others. Many insects are narrowly oligophagous and even sometimes monophagous, which suggests high biodiversity and endemism. The Georgian Red Book of endangered plants lists the species from the BKNP.

With reference to the fauna of the BKNP, no detailed studies have been conducted yet. In the context of biodiversity, insects are an ideal subject for assessing the impact of disturbance on ecosystem composition and dynamics [Ignacio et al., 2001]. Furthermore, insects may serve as "test organisms" for comparing disturbed and undisturbed sampling sites because of the functional relationships among species and the high abundance in many taxa [Zilihona & Nummelin, 2001]. Carabids were chosen for the current study because they are a diverse insect family, fairly easy to identify to species level, and have important ecosystem functions. They are also strongly linked with plant associations and reflect the biodiversity of the studied area [Magura et al., 2003]. Carabid beetles are appropriate organisms to study the impacts of habitat alteration because they are sufficiently varied both taxonomically and ecologically; moreover, they are abundant and their ecology and systematics are relatively well known [Lövei & Sunderland, 1996; Niemela et al., 2000; Allegro & Sciaky, 2002].

Data on the carabid fauna of the Caucasus are scarce [Kryzhanovskij & Ter-Minasian, 1958]. Kryzhanovskij et al. (1995) recorded 1194 species for the Great Caucasus and 408 species for the Lesser Caucasus with 647 and 49 endemic species respectively. Six hundred ten ground beetle species are in the Georgian carabid beetle checklist [Reck & Chaladze, 2004]. The studies above suggest that investigations on carabids in Georgia are fragmentary, and mainly faunistic in nature.

The aim of this survey was to study the carabid fauna of five habitats and find out the difference between biodiversity caused by different types of vegetation, assuming carabids can be used as surrogate indicators of biodiversity in these habitats. The results of this study therefore

have implications for the conservation management of the area. The current study can also serve as a basis for future long term investigations on the biodiversity conservation processes of BKNP, and can be used as a case-study in future investigations.

Materials and Methods

BKNP covers more than 75,928 hectares [Abuladze et al., 2001], which amounts to nearly 1% of the territory of Georgia. The study area was divided into five sampling areas (Fig. 1) with different plant associations representing all main habitat types of BKNP. The Baniskhevi site is a mesophilic valley, where forests of *Fagus* and *Picea* are characteristic plants for this habitat. One of the peculiarities of the Baniskhevi gorge is *Alnus*. The site also features *Carpinus, Castanea, Fraxinus, Acer* and *Tilia*. In Baniskhevi gorge forests, Georgian oak (*Quercus iberica*) is very scarce. The undergrowth of this habitat consists of *Rhododendron, Prunus, Ilex, Rhamnus* and other Colchis shrubs. The ground is covered by ferns [Abuladze et al., 2001]. Coordinates of this site are: N 41.88225, E 43.356077 and the elevation is 900 m. In dry valley habitat - Kvabiskhevi, the influence of the western humid climate is minor. The site is characterized by steep, erosive and rocky slopes and mountains. *Quercus* spp. dominate in this habitat. Mountain xerophytes such as *Carpinus* and *Cotinus* are common. *Pinus, Picea* and *Carpinus* are also mixed in some places. The coordinates are N 41.7968, E 43.23878 and the elevation is 988 m. The Likani 1 coniferous forest is a mixture of *Pinus, Picea* and *Abies*. The coordinates are N 41.83167, E 43.32349 and the elevation is 998 m; Likani 2, mixed forest, coordinates are N 41.83004 and E 43.30601, with an altitude of 1212 m; and Bairagebis seri, subalpine meadow, where subalpine pine forest with undergrowth of *Juniperus* is predominant. *Bromopsis variegate, Chamaenerion angustifolium, Dentaria bulbifera, Digitalis ferruginea, Euphorbia* cf. *glaberrima, Gadellia lactiflora, Hypericum* sp., *Leucanthemum vulgare, Paeonia steveniana, Petasites albus, Phleum phleoides, Pimpinella rhodantha, Primula* sp., *Sanicula europaea, Scabiosa caucasica, Senecio othonnae, Rosa sp., Urtica dioica* are also present. Scarce groves of Picea are also represented. Coordinates are N 41.84043 and E 43.27454 and the altitude is 1839 m.

Specimens were collected in pitfall traps from April 15 to September 25, 2008. Traps were checked monthly. At all sampling sites, 10 pitfall traps were set with a distance between them of 15-20 m. The pitfall traps consisted of circular pots, each 8.5 cm in diameter and 12 cm deep. They were dug into the soil so that the opening was leveled with the soil surface. At the beginning of the study dry traps were used, but a month later it was necessary to modify the methodology, as specimens were being damaged. Two percent formaldehyde solution was added to the traps to avoid damage by the insects themselves, and other animals such as mice, shrews and lizards. Accidental death of small mammals is not regulated by the Georgian law on wild life [Law on wild life, 1997]. After collection, all material was preserved in 75% ethanol. In the laboratory, two drops of acetic acid were added to soften the material for 30 minutes before pinning or mounting on cards. For species determinations different keys and web sources were used [Medvedev, 1965; Borror et al., 1989; Pickering, 2009; Bartlet, 2009; Anonymous, 2009]. Identifications were made by second author.

The diversity index was calculated with the Shannon – Wiener equation:

$$H' = -\sum pi \ln(pi)$$

where *pi* is the proportion of individuals found in the '*i*th' family.

Species richness indices were calculated with Margalef's diversity index equation:

$$D_{mg} = \frac{(S-1)}{\ln N}$$

where S is a number of recorded species and N is a total number of individuals in the sample.

Dominance measures were calculated with the Simpson index equation:

$$l = \sum ni(ni-1)/N(N-1)$$

where *l* is the Simpson index, *ni* is the number of individuals in each of the species and N is the total number of individuals [Magurran, 2005]. The indices were calculated using the Heiman (2004) computing program.

To estimate the total species richness of each site from the abundance data, the Chao 1 equation was used:

$$S_{Chao1} = S_{obs} + \frac{F_1^2}{2F_2}$$

where S_{obs} = the number of species in the sample; F_1 = the number of observed species represented by a single individual (singletons); and F_2 = the number of observed species represented by two individuals (doubletons) [Magurran, 2005].

To estimate the absolute number of species at all sites, the Chao 2 equation was employed:

$$S_{Chao2} = S_{obs} + \frac{Q_1^2}{2Q_2}$$

where Q_1 = the number of species that occur in one sample only (unique species) and Q_2 = the number of species that occur in two samples [Magurran, 2005].

The similarity coefficient was calculated with the Jaccard equation:

Cj=j/(a+b-j)

where a is the number of species at site A, b is the number of species at site B, and j is the number of species found at both sites.

Indicator Value (IndVal) for each species, as a percentage, was calculated according to the method of Dufrene & Legendre (1997).

The specificity measure was calculated as $A_{ij} = Nindividuals_{ij}/Nindividuals_i$ where $Nindividuals_{ij}$ is the mean number of species *i* across sites of group *j*, and $Nindividuals_i$ is the sum of the mean number of individuals of species *i* over all groups;

The fidelity measure was calculated as $B_{ij} = Nsites_{ij}/Nsites_j$ where $N\ sites_{ij}$ is the number of sites in cluster (habitat) *j* where species *i* is represented, and $N\ sites_j$ is the total number of sites in the cluster.

The indicator value for species I in the cluster (habitat) was then calculated as a percentage by using $Ind\ Val_{ij} = A_{ij} \times B_{ij} \times 100$.

The Biodiversity Professional computing program was used to build curves for species abundance and richness, and for cluster analyses [McAleece, 1997].

Results and Discussion

Collections at five sites resulted in 891 data-based specimens which were all identified to species level. The carabid compositions of 5 habitats at BKNP are shown in Table 1. Twenty seven

species were collected during the survey. The greatest diversity of carabids was found at site Bairagebis seri (22 species) (Table 1).

Table 1. Relative abundance of carabids and basic site-by-site descriptive characters at five sites in Borjom-Kharagauli National Park, Georgia.

Species	Sampling sites				
	Baniskhevi	**Kvabiskhevi**	**Likani 1**	**Likani 2**	**Bairagebis seri**
Agonum sp.	0	0	0	0	0.036
Amara sp1	0	0	0.008	0	0.01
Amara-sp2	0	0	0	0	0.002
Badister bipustulatus	0	0	0	0	0.002
Calathus sp.	0	0	0.025	0	0.014
Calathus reflexicollis	0.05	0	0	0	0
Carabus armeniacus	0.36	0.42	0.46	0.8	0.045
Carabus cribratus	0	0	0	0	0.01
Carabus ibericus	0.2	0.075	0.18	0.022	0.045
Carabus koenigi	0	0	0	0	0.079
Carabus puschkini	0.03	0.12	0.11	0.015	0.012
Carabus septemcarinatus	0.2	0.12	0.19	0.13	0.007
Carabus-sp1	0	0.04	0	0	0.007
Carabus-sp2	0	0	0	0	0.002
Cicindela germanica	0.03	0	0	0	0
Cychrus aeneus	0.05	0.15	0.008	0.007	0.014
Harpalus aeneipennis	0	0	0	0	0.019
Harpalus sp.	0	0	0.008	0	0
Harpalus rufipes	0.05	0	0	0	0
Laemostenus sericeus	0.03	0.075	0	0	0.038
Laemostenus suramensis	0	0	0	0.022	0
Pterostichus (Myosodus) sp.	0	0	0	0	0.053
Pterostichus armenus	0	0	0	0	0.13
Pterostichus chydaeus	0	0	0	0	0.385
Pterostichus sp.	0	0	0	0	0.005
Tachyta nana	0	0	0	0	0.002
Thermoscelis insignis	0	0	0	0	0.08
H'	1.8	1.67	1.44	0.72	2.21
E	0.52	0.59	0.42	0.25	0.24
D_{mg}	1.96	1.85	1.46	0.89	3.48
l	0.21	0.24	0.3	0.66	0.19
Mean Individuals	2.143	0.929	4.25	9.571	14.929
Standard Deviation	4.866	2.276	11.727	40.652	31.285
Total Individuals	60	26	119	268	418
Total Species	9	7	8	6	22
Unique Species	3 (33%)	0 (0%)	1 (12.55)	1 (16.7%)	13(59.1%)

H'- Shannon-Wiener diversity Index; *E* – Shannpn-Wiener Eveness
D_{mg} - Margalef's diversity Index; *l* – Simpson dominance Index

The estimated absolute number of species at all sites was 36, which means that 25% of Carabidae fauna is still uncollected at the BKNP. The genus *Carabus* spp. was the most abundant genus at all sites and the genus *Cychrus* was also recorded at all sites, but in low numbers.

The basic descriptive characters for the five sites are given in Table 1 and Fig.2. In Fig.2, k-dominance sites are ranked by cumulative abundance of species. The curve corresponding to site Likani 2 is located above the other curves, which means that this community is the least diverse.

The highest abundance of carabids was harbored at site Bairagebis seri (47%), followed by site Likani 2 (30.1%), site Likani 1 (13.3%), site Baniskhevi (6.7%) and site Kvabiskhevi (2.9%). The species richness indices and rarefaction curves are shown in Fig. 3, with site Bairagebis seri (3.48) being the most diverse and site Likani 2 (0.89) being the least diverse. Curves show that abundance and diversity indices are correlating with each other.

The lowest Shannon-Wiener diversity index was found at site Likani 2 (0.72), Margalef gave similar results (Table 1). Site Bairagebis seri does not cluster with any of the others sites in the cluster analysis (Fig. 4). The highest percentage similarity index (0.63) was between sites Kvabiskhevi and Likani 2 (Baniskhevi/Kvabiskhevi – 0.6; Baniskhevi/Likani 1 – 0.42; Baniskhevi/Likani 2 – 0.5; Baniskhevi/Bairagebis seri - 0.24; Kvabiskhevi/Likani 1 – 0.5; Kvabiskhevi/Bairagebis seri – 0.21; Likani 1/Likani 2 – 0.55; Likani 1/Bairagebis seri – 0.3; Likani 2/Bairagebis seri – 0.22). The lowest similarity indices were between Likani 2/Bairagebis seri (0.22) and Kvabiskhevi/Bairagebis seri (0.21).

Distributions and abundance of Carabidae species were different between sampling sites. Agonum sp., Amara sp.2, Badister bipustulatus, Carabus cribratus, C. koenigi, C. sp.1, Harpalus aeneipennis, Pterostichus armenus, P. chydaeus, P. sp., P (Myosodus) sp., Tachyta nana and Thermoscelis insignis were recorded only at site Bairagebis seri; Calathus reflexicollis, Cicindela germanica and Harpalus rufipes only from site Baniskhevi; and Harpalus sp. and Laemostenus suramensis only from sites Likani 1 and Likani 2 respectively.

The species with the highest indicator value (IndVal)(66.9) was *Carabus armeniacus* for site Likani 2 (Table 2). *Carabus puschkini* and *Laemostenus sericeus* also had high indicator values for sites Likani 1 (48) and Bairagebis seri (48), respectively. The indicator value of *Carabus septemcarinatus* for site Likani 2 was 46.8, of *Cychrus aeneus* for site Bairagebis seri was 37.5 and of *Carabus ibericus* for sites Likani 1 and Bairagebis seri was 36 and 31, respectively.

During our survey eight species endemic to the Caucasus were recorded: Pterostichus (Myosodus) lacunosus, Carabus armeniacus, C. pushkini, C. septemcarinatus, C. ibericus, Laemostenus sericeus, Cychrus aeneus and Thermoscelis insignis [Kryzhanovskij et al., 1995]. However, only Carabus armeniacus, C. pushkini, C. septemcarinatus and C. ibericus had a significant Indicator Value for sites. Because of Laemostenus sericeus and Cychrus aeneus also had high indicator values these species can be also used as indicators for their respective habitats.

Our study revealed 27 carabid species collected by pitfall traps. The present study revealed that the Bairagebis seri site (subalpine meadow) was an outstanding sampling site for carabid diversity. This may be attributable to the influence of the high diversity grasslands [Abuladze et al., 2001]. The lowest abundance was found at site Kvabiskhevi (dry valley) which may be explained by the close proximity of local inhabitants, high touristic activity and also its eroded and rocky slopes. The park is still used for agriculture and grazing, and human influence continues to negatively affect the fauna. The evidence for this assertion is that only 2.9 % of all carabid samples were collected there.

For the species found that have the highest indicator value for different sites, *Carabus armeniacus*, *Carabus puschkini*, *Laemostenus sericeus, Carabus septemcarinatus, Cychrus aeneus*, *Carabus ibericus*, long-term observations are needed to determine what processes are occurring in those particular habitats.

All endemic species for Caucasus: (Myosodus) sp., Thermoscelis insignis, Carabus armeniacus, Carabus pushkini, Carabus septemcarinatus, Carabus ibericus, Laemostenus sericeus and Cychrus aeneus also require long-term observation and conservation measures. No data about the IUCN status of species represented in our study is known; this may be determined after future studies on the group.

Table 2. Indicator Value for each species

Species	Sampling sites				
	Baniskhevi	Kvabiskhevi	Likani 1	Likani 2	Bairagebis seri
Agonum sp.	0	0	0	0	20
Amara sp1	0	0	4	0	16
Amara-sp2	0	0	0	0	20
Badister bipustulatus	0	0	0	0	20
Calathus sp.	0	0	13	0	26.8
Calathus reflexicollis	20	0	0	0	0
Carabus armeniacus	6.5	3.4	17	66.9	5.9
Carabus cribratus	0	0	0	0	20
Carabus ibericus	19.7	3.2	36	10	31
Carabus koenigi	0	0	0	0	20
Carabus puschkini	7.4	11.1	48	14.8	18.5
Carabus septemcarinatus	15.6	3.9	29.9	46.8	3.9
Carabus-sp1	0	13.2	0	0	30
Carabus-sp2	0	0	0	0	20
Cicindela germanica	20	0	0	0	0
Cychrus aeneus	18.8	25	6.25	12.5	37.5
Harpalus aeneipennis	0	0	0	0	20
Harpalus sp.	0	0	20	0	0
Harpalus rufipes	20	0	0	0	0
Laemostenus sericeus	6	6	0	0	48
Laemostenus suramensis	0	0	0	20	0
Pterostichus (Myosodus) sp.	0	0	0	0	20
Pterostichus armenus	0	0	0	0	20
Pterostichus chydaeus	0	0	0	0	20
Pterostichus sp.	0	0	0	0	20
Tachyta nana	0	0	0	0	20
Thermoscelis insignis	0	0	0	0	20

Fig.1. The location of carabid sampling sites in BKNP, Georgia

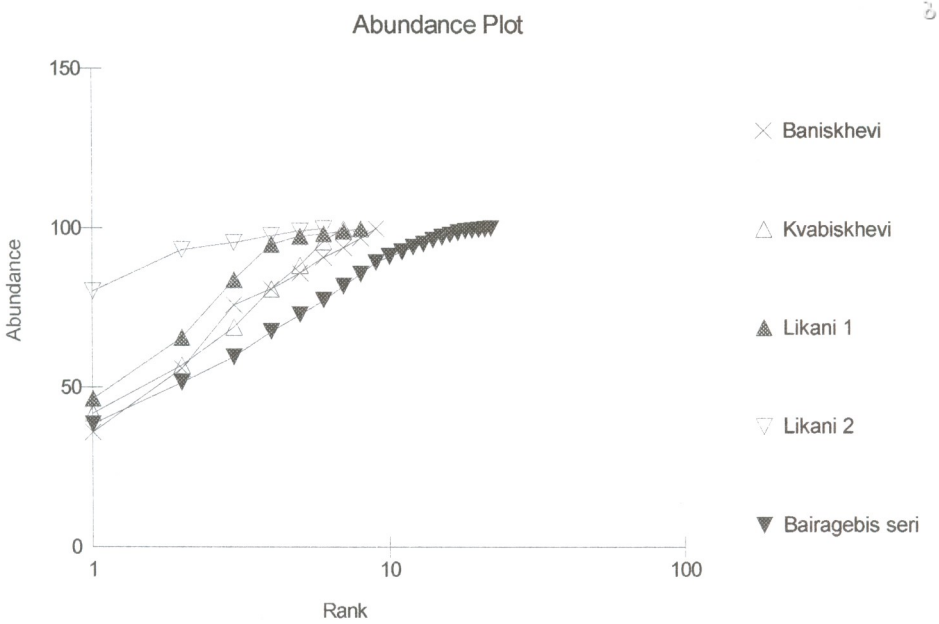

Fig. 2. Diagram of k-dominance of carabids at the study sites.

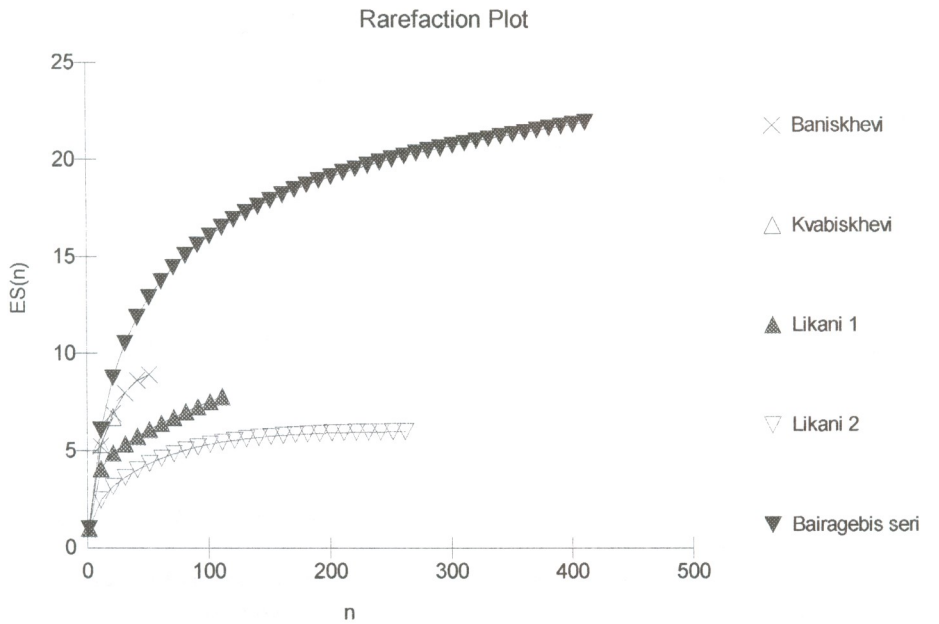

Fig. 3. Sample-based rarefaction curves for the carabids of the study sites.

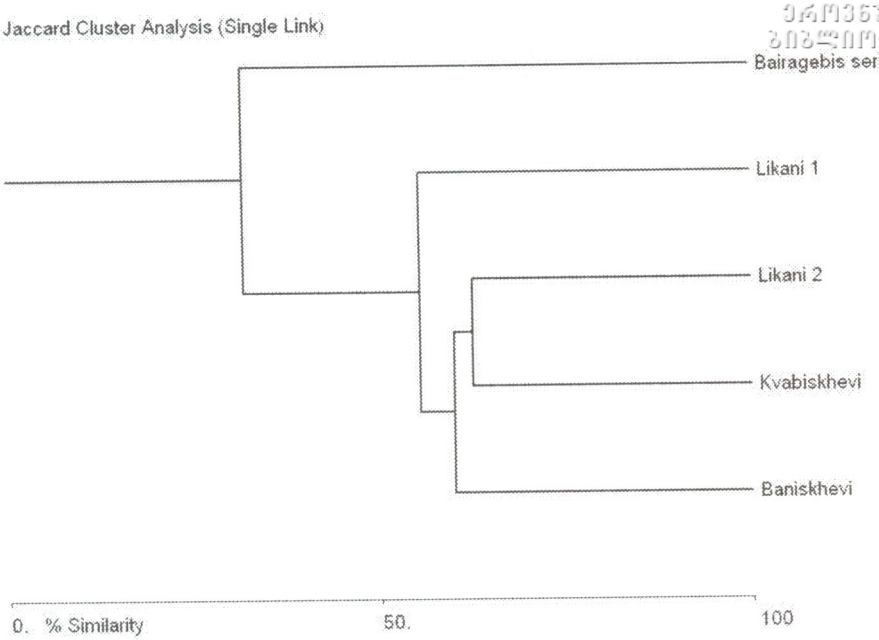

Fig. 4. Cluster analysis of carabid fauna similarity among sampling sites at the species level

It is generally accepted that species with small population sizes suffer the most through habitat destruction or disturbance. Tilman et al. (1994) reported that destroying an additional 1% of a habitat caused eight times more extinction of species with small populations than in similar sized disturbed habitats. We therefore recommend further long-term surveys in the BKNP using other coleopteran groups as taxonomic indicators for assessing natural and human induced processes in the park. Enhanced conservation measures, including monitoring, would assist the survival of rare and endangered species and populations. To facilitate that process, each recovery measure should improve habitat conditions and increase biodiversity. Conservation efforts and monitoring in the study area could employ selected coleopteran groups as taxonomic indicators to help adjust mitigation measures for habitat damage.

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ბორჯომ-ხარაგაულის ეროვნული პარკის ბზუალების მრავალფეროვნებისა და შემადგენლობის პირველადი მონაცემები საქართველოში

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რეზიუმე

კვლევა ჩატარდა საქართველოში, ბორჯომ-ხარაგაულის ეროვნული პარკის ბზუალების მრავალფეროვნებაზე. შერჩეული იქნა 5 განსხვავებული მცენარეთა ასოციაციის წერტილი, რომელიც მოიცავდა ბორჯომ-ხარაგაულის ეროვნული პარკის ჰაბიტატების ძირითად ტიპებს: მეზოფილური ველი - ბანისხევი; მშრალი ველი - ქვაბისხევი, ამ წერტილზე დასავლეთის ნესტიანი კლიმატის გავლენა მინიმალურია; წიწვოვანი ტყე – ლიკანი 1; შერეული ტყე – ლიკანი 2; და ბოლოს სუბალპური მდელოები - ბაირაღების სერი.

ოცდაშვიდი სახეობა იყო რეგისტრირებული კვლევის პერიოდში. Carabid-ები ყველაზე მრავალრიცხოვანი იყო ბაირაღების სერზე, რომელიც ხასიათდებოდა ბალახოვნების მაღალი მრავალფეროვნებით. შეგროვილი ეგზემპლიარების ორმოცდაშვიდი პროცენტი სწორედ ამ წერტილში იყო შეგროვებული. კვლევამ აჩვენა, რომ ეს წერტილი წარმოადგენდა განსაკუთრებულ მიკროჰაბიტატს ბზუალების ფაუნისათვის. ცხრამეტი სახეობა აღრიცხული იყო მხოლოდ ამ წერტილში. *Carabus armeniacus, C. ibericus, C. puschkini, C. septemcarinatus* და *Cychrus aeneus* განისაზღვრენ, როგორც ინდიკატორი სახეობები. ბზუალების სახეობრივი სიმჭიდროვე განსხვავებული იყო განსხვავებულ ჰაბიტატებში.

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Paleobiology

THE FLORA AND VEGETATION OF EASTERN GEORGIA IN THE SARMATIAN

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Abstract

The full sections of Sarmatian deposits of Eastern Georgia were studied by palynological method. The landscape-phytocenological analysis of material reflects the changes of ecological-systematical composition of flora and allows to follow the evolution of vegetation in Kartli and Kakheti depending on climatic fluctuations.

Key words: Eastern Georgia, Sarmatian, palynology.

Introduction

Sarmatian deposits are widely distributed on the territory of Eastern Georgia and are divided into three substages: Volhinian (Lower), Bessarabian (Middle) and Khersonian (Upper). The Lower and Middle Sarmatian are built by marine deposits. The thick series of continental deposits of Natskhorian suit, which has a wide distribution and is the part of most syncline and anticline folds, belongs to the Upper Sarmatian in Kartli.

The Upper Sarmatian on the territory of Kakheti is different. In the North-Western part of region it is represented by continental deposits of Eldarian suit, with tests of freshwater and terrestrial gastropoda. Towards the south-east the clays of lower part of Eldarian suit are replaced by marine sediments with fauna of Mactra [Buleishvili, 1960; Koiava, 2006]. The presence of "marine suit" is confirmed also by micropaleontological investigations of core material from boreholes [Maissuradze et al., 2006].

Materials and Methods

Until recently the most of our knowledge on the Sarmatian flora of Eastern Georgia was based on macrobotanical data [Uznadze, 1965; Chelidze, 1987]. Whole material is connected with deposits of the Lower and Middle Sarmatian. By palynological method only some core samples from Central part of the Kartli depression were studied [Mchedlishvili, Mchedlishvili, 1953]. In spite of poorness of fossil material (28 forms), the palynological assemblages, typical for three substages of the Sarmatian were distinguished.

Now the rich body of palynological material was collected from full sections of Sarmatian deposits on the territory of Kartli (sections Nadarbazevi, Aragvi) and Kakheti (sections Davit

Gareji, Gombori). The palynological assemblages were interpreted by landscape-phytocenological method [Borzenkova, 1992]. The whole composition of palynoflora was divided into some ecological groups of plants: the conifers of temperate climate (Abies, Picea, Tsuga); subtropical and warm-temperate conifers (Podocarpus, Dacrydium, Cathaya, Keteleerua Cedrus); warm-temperate leaf-bearing plants (Carya, Juglans, Fagus, Quercus, Carpinus, Zelkova and others); subtropical leaf-bearing plants (the representatives of families Myricaceae, Fagaceae, Lauraceae, Hamamelidaceae, Araliceae, Icacinaceae, Alangiaceae, Arecaceae); subtropical ferns (Anemia, Lygodium, Mohria, Cyathea, Dicksonia, Cleichenia, Pteris, Polypodium); xerophylous plants of woodless areas (the representatives of families Chenopodiaceae, Asteraceae, Poaceae and others). Two palynological diagrams were built. One for Kartli on the basis of material from Nadarbazevi section (Fig.1) and second - for Kakheti based on the palynological material from Gombori section (Fig.2). The curves of these diagrams reflect the changes in composition in mentioned above groups during the Lower, Middle and Upper Sarmatian. The curve of pine, as an intrazonal plant and index of humidity, is given separately.

As a whole based on palynological data in the composition of Sarmatian flora of Eastern Georgia nearly 200 elements belonging to 137 genera and 93 families, were determined: *Sphagnum* sp., *Lycopodium serratum* Thumb., *Lycopodium* sp., *Selaginella* sp., *Selaginella fusca* N. Mchedl., *Botrychium* sp., *Ophioglossum* sp., *Osmunda cinnamomea* L., *Osmunda* sp., *Schizaea* sp., *Schizaeaceae* gen.indet., *Anemia* sp., *Mohria* sp., *Lygodium digitatum* Presl., *Lygodium japonicum* Sw., *Lygodium* aff. multivallatum (W.Kr.) Ram., *Lygodium* sp., *gramma* sp., *Pteridacidites longifoliiformis* Sh. et St., *Pteridacidites venustaeformis* Sh. et St., *Pteridacidites* aff. verus (N. Mchedl.) Sh. et St., *Pteridacidites* aff. vittatoides Shat. Stuch., *P. guriensis* Sh. et St., *P. grandifoliiformis* St. et Sh., *P. boerzoenyensis* St. et Sh. (Nagy), *P. dentatiformis* Sh. et St., *Pteridacidites* sp., *Pteris* sp., *Marsilea* sp., *Anogramma* sp., *Onychium* sp., *Pityrogramma* sp., *Clavifera* aff. *tuberosa* Bolch., *Clavifera* sp., *Gleichenia* sp., *Gleicheniaceae* gen. indet., *Polypodium aureum* L., *Polypodium verrucatum* Ram., *Polypodium* sp., *Polypodium plicenicum* Ram., *Verrucatosporites histiopteroides* W. Kr., *Pyrrosia* sp., *Polypodiaceae* gen.indet., *Hymenophyllum* sp., *Cibotium* sp., *Dicksonia spanditocincta* Purc., *Dicksonia unitotuberata* Purc., *Dicksonia* sp., *Dicksonia reticulata* Purc., *Alsophylla* sp., *Cyathea* sp., *Hemitelia* sp., *Asplenium* sp., *Cystopteris* sp., *Dryopteris* sp., *Microlepia* sp., *Thelypteris* sp., *Woodsia* sp., *Polystichum* sp., *Ginkgo* sp., *Dacrydium* sp., *Podocarpus* sp., *Phyllocladus* sp., *Araucaria* sp., *Abies alba* Mill., *Abies ciliticaeformis* N. Mchedl., *Abies nordmanniana* (Stev.) Spach., *Abies* sp., *Cathaya* sp., *Cedrus deodara* Loud., *Cedrus sauerae* N. Mchedl., *Cedrus* sp., *Keteleeria caucasica* Ram., *Picea complanataeformis* N. Mchedl., *Picea minor* N. Mchedl., *Picea* sp., *Pinus rjabinini* Palib., *Pinus* sp., *Pseudolarix* sp., *Pseudotsuga* sp., *Tsuga* aff. *diversifolia* (Maxim.) Mast., *Tsuga* aff. *canadensis* (L.) Carr., *Tsuga* aff. *pattoniana* Engelm., *Tsuga* sp., *Pinaceae* gen.indet., *Sciadopitys* sp., *Cryptomeria* sp., *Cunninghamia* sp., *Sequoia* sp., *Metasequoia* sp., *Sequoiadendron* sp., *Taxodium* sp., *Taxodiaceae* gen. indet., *Juniperus* sp., *Libocedrus* sp., *Juniperus* sp., *Cupressaceae* gen. indet., *Ephedra* sp., *Comptonia* sp., *Myrica* sp., *Myrica* aff. *notabilis* Gladk., *Myricaceae* gen. indet., *Carya aquatica* (Michx.) Nutt., *Carya cordiformis* (Wangh.) C. Koch, *Carya* sp., *Carya ovata* Mill., *Carya* aff. *glabra* (Mill.) Sveet, *Engelhardia* sp., *Juglans cinerea* L., *Juglans regia* L., *Juglans* sp., *Platycarya* sp., *Pterocarya pterocarpa* (Michx.) Kunth., *Pterocarya* aff. *stenoptera* DC, *Pterocarya* sp., *Juglandaceae* gen.indet., *Salix* sp., *Alnus* sp., *Betula* sp., *Carpinus betulus* L., *Carpinus caucasica* Grossh., *Carpinus* orientalis Mill., *Carpinus* sp., *Corylus* sp., *Castanea* sp., *Castanopsis* sp., *Lithocarpus* sp., *Fagus* sp., *Quercus* sp., *Celtis* sp., *Ulmus foliacea* Gilib., *Ulmus* sp., *Zelkova carpinifolia* (Pall.) Dipp., *Zelkova serrata* (Thinb.) Makino, *Zelkova* sp., *Ulmaceae* gen.indet., *Eucommia ulmoides* Oliv., *Ficus* sp., *Moraceae* gen.indet, *Polygonaceae* gen. indet., *Caryophyllaceae* gen.indet., *Chenopodiaceae* gen. indet., *Liriodendron* sp., *Magnolia megafigurata* (Krutsch) comb. nov Ram., *Magnolia grandiflora* L., *Magnolia*sp., Annona sp., *Cinnamomum* sp.,

Laurus sp., *Lauraceae* gen. indet., *Ranunculus* sp., *Berberidaceae* gen. indet., *Menispermum* sp., *Nuphar* sp., *Nymphaea* sp., *Nymphaeaceae* gen. indet., *Brassicaceae* gen. indet., *Aristolochia* sp., *Papaver* sp., *Platanus* sp., *Corylopsis* sp., *Disanthus* aff. *cercidifolia* Maxim., *Disanthus* sp., *Fothergilla* sp., *Hamamelis* sp., *Parrotia* sp., *Sycopsis* sp., *Sycopsis colchica* Ram., *Liquidambar* sp., *Liquidambar styraciflua* L., *Hamamelidaceae* gen.indet., *Cercidiphyllum* sp., *Pyrus theobroma* Ung., *Sorbus* sp., *Rosaceae* gen.indet., *Acacia* sp., *Fabaceae* gen.indet., *Geranium* sp., *Rhus* sp., *Acer* sp., *Aesculus* sp., Ilex sp., *Icacinaceae* gen.indet., *Euonymus* sp., *Staphylea* sp., *Buxus* sp., *Parthenocissus* sp., *Vitis* sp., *Tilia caucasica* Rupr., *Tilia* aff. *cordata* Mill., *Tilia* aff. *platyphyllos* Scop., *Tilia* sp., *Sterculia* sp., *Elaeagnus* sp., *Viola* sp., *Myrtaceae* gen.indet., *Onagraceae* gen.indet., *Alangium* sp., *Nyssa* sp., *Cornaceae* gen.indet., *Acanthopanax* sp., *Aralia* sp., *Brassaiopsis* sp., *Dendropanax* sp., *Fatsia* sp., *Araliaceoipollenites edmundi* (Pot.) Pot., *Araliaceae* gen.indet., *Apiaceae* gen.indet., *Arbutus guriensis* Uzn., *Rhododendron* sp., *Ericaceae* gen.indet., *Sapotaceae* gen.indet., *Symplocos* sp., *Apocynaceae* gen.indet., *Convolvulus* sp., *Fraxinus* sp., *Oleaceae* gen.indet., *Lonicera* sp., *Viburnum* sp., *Lamiaceae* gen.indet., *Plantago* sp., *Valeriana* sp., *Knautia* sp., *Scabiosa* sp., *Dipsacaceae* gen.indet., *Achillea* sp., *Artemisia* sp., *Cichorium* sp., *Asteraceae* gen.indet., *Liliaceae* gen.indet., *Poaceae* gen. indet., *Nipa* sp., *Arecaceae* gen.indet., *Pandanus* sp., *Sparganium* sp., *Typha latissima* A.Br., *Typha* sp., *Fupungopollenites wackersdorfensis* (Thiele-Pfeiffer) Liu Geng-wu., *Fupingopollenites minutus* Liu Geng-wu.

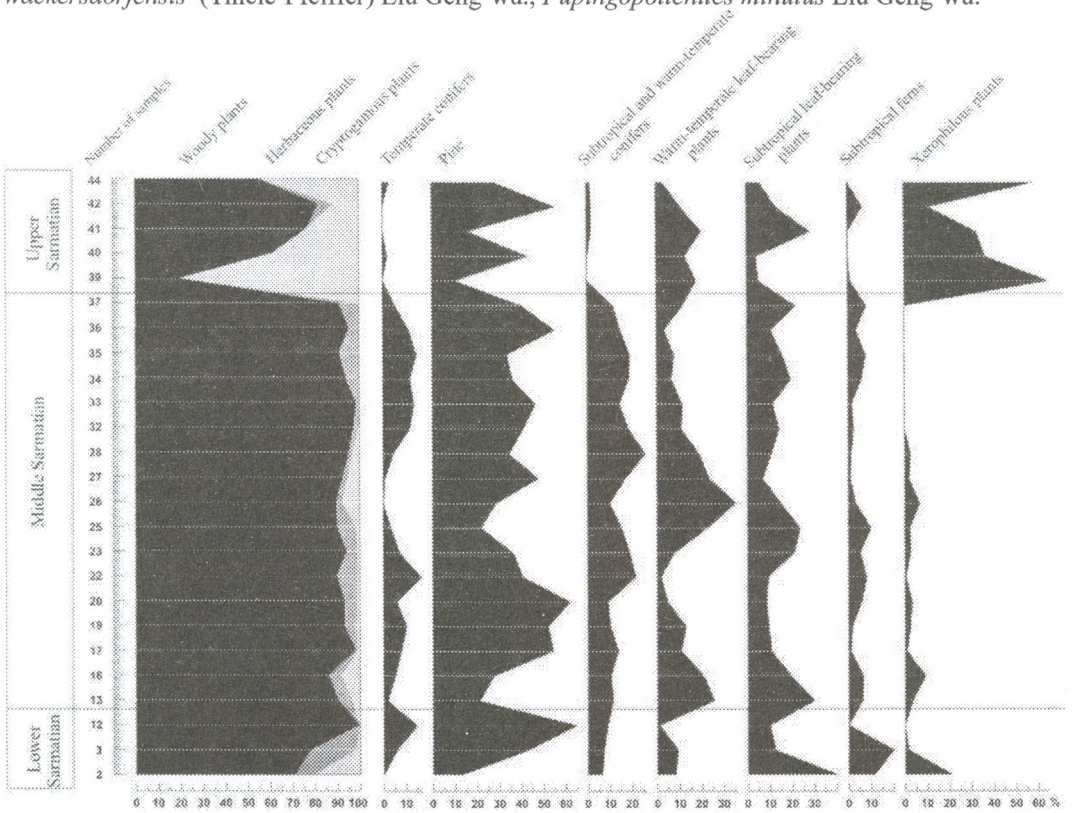

Fig.1. Fluctuations in pollen percentages indicating changes in composition of separate ecological groups of plants in the Sarmatian of Kartli (section Nadarbazevi)

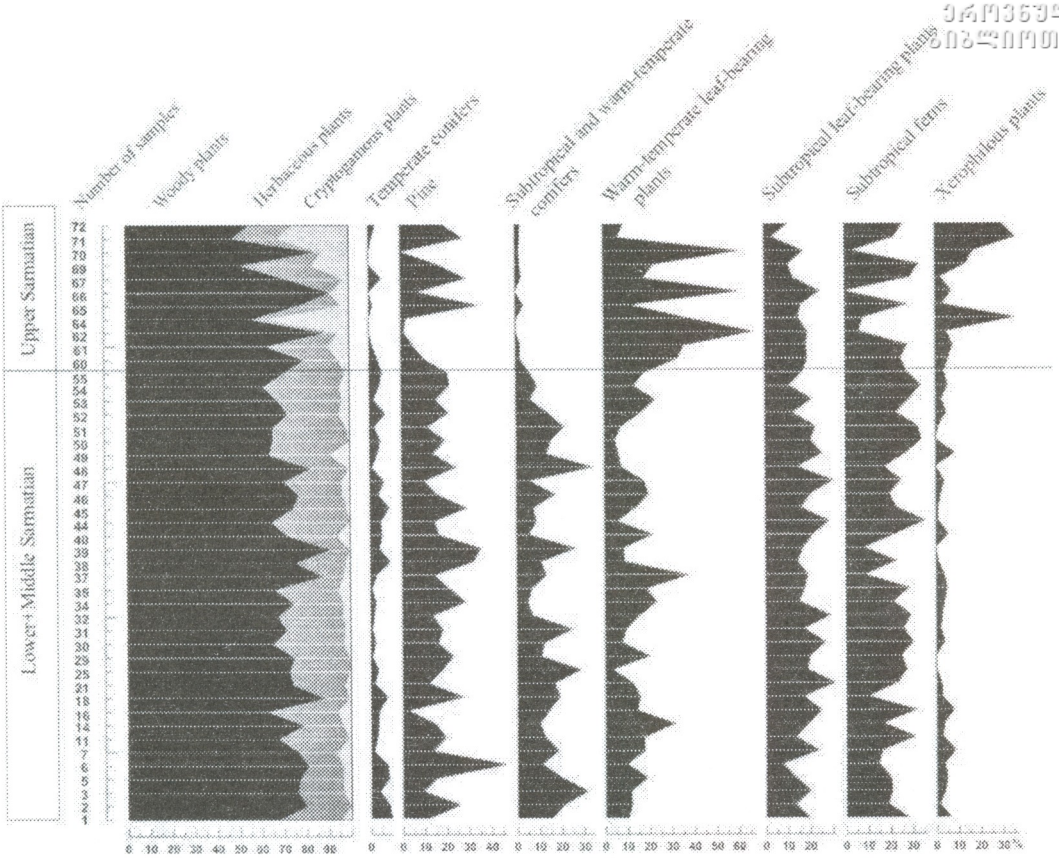

Fig.2. Fluctuations in pollen percentages indicating changes in composition of separate ecological groups of plants in the Sarmatian of Kakheti (section Gombori)

Results and Discussion

Judging from the palynological assemblages and macroremains of plants the main formation on the territory of Eastern Georgia during the Early and Middle Sarmatian was the polydominant forest.

The more termophilous and hygrophylous plants inhabited in the littoral zone and in lower mountain belt. The components of forest were representatives of families Lauraceae, Araliaceae, Sapotaceae, Icacinaceae, Arecaceae. The lower layer of forest formed the arborescent and grassy ferns: Dicksonia, Cyathea, Lygodium, Anemia, Pteris, Polypodium. Among these forms the last genera predominated. The components of riparian forest were Liquidambar, Nyssa, Pterocarya, Ulmus, Carya and some Hamamelidaceae.

On the territories situated far from the accumulation basin dominated the deciduous forest. In its composition were: Quercus, Castanea, Fagus, Juglans, Carpinus, Zelkova, Platanus, Tilia. The leaf-bearing plants were mixed with gymnosperms: Ginkgo, Dacrydium, Podocarpus, Cathaya, Keteleeria, Cedrus.

The upper levels of relief were occupied by Abies, Picea Tsuga. The area of dark conifer forest was small in comparison with polydominant formation. The pine was the component of different cenosis and occupied the big territories.

The palynological assemblages of the Lower and Middle Sarmatian deposits of Kartli reflect the vegetation, which developed in conditions of subtropical climate with low humidity. Somewhat other situation was on territory of Kakheti. Here the role of pine was smaller and the part of thermophilous and hygrophilous elements of flora in composition of polydominant forest was much greater. Among ferns such forms as Mohria, Anemia, Lygodium, Gleichenia, dominated.

The boundary between the Middle and Upper Sarmatian in Kartli is led by appearance of conglomerate layer, indicating the changes in process of sedimentation [Koiava. 2006]. In all sections studied by palynological method the deposits of Khersonian substage distinguished by composition of pollen assemblages, which reflect the changes in vegetation. In the Late Sarmatian the area of forest formation reduced and the territory of woodless significantly increased. All these phenomena more distinctly were manifested in Kartli and in smaller degree in Kakheti, on the plot near Gombori, which distinguished by local microclimate conditions. Nevertheless even the part of xerophilous plant in composition of vegetational cover was increased here.

Conclusion

The characteristic sign of Sarmatian vegetation of Eastern Georgia was the predominance of subtropical and warm-temperate plants. In composition of cenosis the hard-leaved and moist-subtropical components were represented. Already in the Sarmatian the differences between vegetation of Kartli and Kakheti are observed. In Kartli the development of vegetation in the Early and Middle Sarmatian was gone in conditions of low humidity, that in the Late Sarmatian and in the following stretches of Neogene led to full succession of forest formations by woodless areas [Mchedlishvili, Mchedlishvili, 1953; Meladze, 1967]. In Kakheti the changes in character of vegetation on the boundary of the Middle and Upper Sarmatian was not so sharp, especially in the northern part of the region.

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აღმოსავლეთ საქართველოს სარმატული დროის ფლორა და მცენარეულობა

შატილოვა ი.[1], კოკოლაშვილი ი.[2]

[1]საქართველოს ეროვნული მუზეუმი, პალეობიოლოგიის ინსტიტუტი
[2]საქართველოს ტექნიკური უნივერსიტეტი

(მიღებულია 12.10.2011)

რეზიუმე

აღმოსავლეთ საქართველოს სარმატული ნალექების სრული ჭრილი შესწავლილი იქნა პალინოლოგიურად. ლანშაფტურ-ფიტოცენოლოგიური მეთოდის გამოყენებამ საშუალება მოგვცა აღგვედგინა ცვლილებები ფლორის შემადგენლობაში და თვალი გვედევნებინა მცენარეულობის განვითარებისათვის ქართლისა და კახეთის ტერიტორიაზე კლიმატის ფლუქტუაციებთან კავშირში.

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DYNAMICS OF DIVERSITY OF NEOGENIC VERTEBRATES IN KAKHETI (EASTERN GEORGIA) AND ADJACENT AREAS

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Abstract

Fauna of fossil vertebrates plays a decisive role in the matter of establishing geological age of continental sediments and comparison of sediments of corresponding age of adjacent regions. Paleontological material gives us indisputable opportunity to follow evolution of paleogeographic picture of past and separate corresponding stages of environment changes. It is also clear, that number of theoretical and practical issues of evolution is clarified on the ground of paleontological investigations.

Unfortunately, Georgia, namely territory of Kakheti does not gratify us with abundance of the sites of vertebrate fauna. Therefore, it is clear, that discovery of a new paleontological sample is always in the focus of paleontologists and geologists.

Key words: Vertebrata, Pliocene, Meotian, Kakheti, Fauna, Agchagilian

The task of presented work is review of faunas of fossil vertebrates of Kakheti, establishment of biostratigraphic values of faunistic complexes, precise definition of stratigraphic levels of geological formation of Kakheti and adjacent regions, and their comparison with geological strata of Mediterranean sea.

Many interesting works were dedicated to the questions of geology, paleobotanics, paleozoology and others [Buleishvili 1960; Burchak-Abramovich, Gabashvili,1945; Gabunia, 1959; Vekua, 1972; Vereshchagin, 1959; Dolidze, 1962; Palibin, 1933; Tsereteli, 1942; Tsiskarishvili, 1987; Meladze, 1967, 1985; Chelidze, 1988; Kvavadze, Vekua 1993]. But as a result of intensive geological-paleontological investigation carrying out at present, much new material is accumulated, essentially filling deficiency of fossil material and defects of our knowledge, which demands new approaches and comprehension.

The raising of Caucasus, which had been lasted in Miocene, caused the formation of land of vast South Caucaus. By paleontological and palynological data, in the second half of Sarmat strengthening of xerophytisation took place, open spaces occupied rather large areas, where Hipparion faunas with their accompanying elements dominated.

At the end of Miocene South Caucasus is already outpost of land of Asia Minor, to the west and the east it borders with Pontus and Caspian basins and to the north with Caucasus ridge. The eastern and partially central parts of this outpost were occupied by the present-day plateau of Iori. Mentioned land is mainly characterized with the spreading of landscape of plateau, which was split by gorges. Paleontological data evidence that in the second half of Sarmat this land was inhabited with rich and diverse fauna of vertebrates, the main core of which is represented by the animals living in the forest. Palynological data also testifies existence of forest tracts in Middle

Sarmat [Gabunia, Chochieva 1959]. Climate would have been rather moist and warm, which does not exclude the existence of open, wide spaces, with the corresponding plant cover, next to forest in this zone [Gabunia, et al., 1996].

Therefore it is natural that in relatively later faunistic complexes of Iori plateau, forms characteristic to steppe and savannah, such as Struthio, Gazella, Oiceros, antelopes, inhabitants of savannah, rodents, reptiles and others are met in increasing frequency [Vekua, 1972].

Strengthening of xerophytisation is especially observed on the plateau of Iori in Plio-Pleistocene. Expansion of plants preferring open spaces occurs in flora [Kvavadze, Vekua 1993]. Fauna of vertebrates, especially mammalians, is changed accordingly. Conditions of relatively moist and warm climate had been developed there finally, which perhaps assisted the penetration of first hominids from Africa into this territory [Gabunia, Vekua, 1993].

Main sites of fauna of vertebrates in Eastern Georgia are concentrated on the plateau of Iori. We should start the history of this fauna with consideration of Miocene Hipparion fauna, which is well represented in South Caucasus on the sites dated by Upper Sarmat. Unfortunately, the initial stage of development of Hipparion fauna – vertebrate fauna of land of Middle Sarmat – is not found in South Caucasus. Sites of Sarmat fauna are defined in Azerbaijan, Armenia and especially Eastern Georgia, namely, on the territory of Kakheti.

Hipparion fauna had dominated over Eurasia, Africa and on the continent of North America almost in the whole period of Mio-Pliocene. Therefore it is clear, what a great importance is attached to the complex study of this fauna, not only for comparison of biostratigraphic and continental strata, but also for the explanation of number of theoretical issues of regularity of development of organic world.

It must be also noted here, that Hipparion fauna of Caucasus, namely of South Caucasus, has a special importance, as its formation was taking place on the crossroad of Eurasian and African continents, which was intensive route of migration for Hipparion faunistic complexes and their separate representatives from Eurasia to Africa and vice versa [Gabunia, Vekua 1968]. Paleontological material enables us to make some attempts for defining not only prohoresis routes and direction of these faunas, but also the rate of spreading of individual mammalians, peculiarities of their adaptation and development of new area for their existence.

We start discussion of Hipparion fauna with the site of Eldar, which is on the territory of Azerbaijan for today, but direct connection of this fauna to Kakheti Miocene fauna is obvious. The age of Upper Sarmat period of Eldar fauna is defined by means of sea-molluscous fauna, confirmed by the analysis of vertebrate fauna as well.

By now, the following are defined in Eldar fauna: Testudo sp., Struthio sp., Phoca procaspica, Ichtitherium hipparionum, Crocuta eldarica, Deinotherium gigantheum, Choerolophodon pentelici, Paleotragus sp., Mirabilocerus eldaricus, Tragocerus aff. leskewitshi, Tragocerus sp., Gazella leilae, Eotraqus (?) martinianus, Microstonyx major, Cervavitus sp. [Gabunia 1959; Gadjiev 1961, 1997).

Hipparion fauna of Eldar is not represented in the form of common biocenose. Forms of various ecological groups are accumulated there, but perhaps the main core of this fauna might have inhabited in the conditions of moist climate. It should be noted as well, that Hipparion of Eldar by its morphological features is closer to Hipparions of Asian origin, and differs from Sarmat Hipparions of Europe essentially [Gabunia 1959].

Site of well-known Hipparion fauna on the territory of David Gareji is located in the south-east, at about 70 km from Tbilisi. Burchak-Abramovich and Gabashvili (1945) discovered two upper teeth (P4 and M1) of primate during the geological-paleontological expedition in the surroundings of Gareji. Researchers caught a likeness of these teeth with anthropoid ape and named it after Gareji (*Udabnopithecus garedziensis*). Later, Georgian scientists considered Udabnopithecus to be the synonym of Driopithecus [Gabunia, et al., 1999]. Discovery of Burchak-

Abramovich and Gabashvili immediately attracted scientists' attention and caused many comments. Main subjects of argumentation were to what species belonged primate and its geological age.

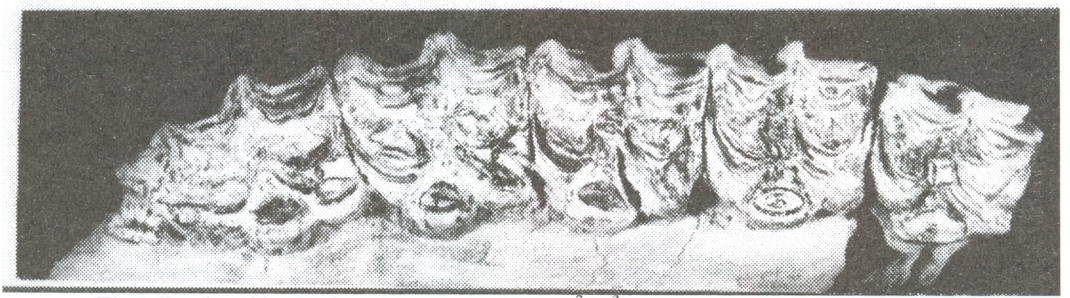

Fig.1. Dzedzvtakhevi. Hipparion elegans Grom. P^2-M^3 occlusal view

At Gareji Monastery clay-sand sediments overlie solid massive sandstones, in which sea Sarmatian fauna of molluscas is found. Quite thick (400 m) continental layer, represented by alternation of coloured clays, sandstones and conglomerates, overlies the stratum with fauna. Coloured layer gradually passes into Shiraki layer, which is transgressively followed by Aghchagil sandstones and clays in turn.

Coloured layer contains remains of fossil animals on two different levels. Difference in composition of two faunas is not observed. By now the following are defined in Gareji fauna: *Testudo eldarica, Udabnopithecus garedziensis, Hystrix* sp., *Hyaena* sp., *Percrocuta gigantean, Hypparion garedzicum, Aceratherium cf. incisivum, Diceros gabunia, Sus major, Tragocerus* sp., *Gazella schlosseri, Palaeotragus rouenii* Tsiskarishvili (1987).

There is no faunistic difference between first and second sites, but the fact, that in the upper faunistic accumulation (desert two) remains of primate-anthropoid ape are found, should be taken into account.

Wangengeim's group carried out paleomagnetic studies of desert sediments and noted that sediments of desert one manifested border of passing from positive magnetization to negative one [Vangengeim et al., 1988], and sediments of desert two are negatively magnetized. On the ground of this data, authors think that sediments of desert one correspond to Upper Sarmat, and sediments of desert two – to the beginning of Meotian. Hence, we conclude that remains of Udabnopithecus were found in Meotian sediments, and it turned to be the youngest geologically among similar primates at the same time.

According to remains of fauna and flora discovered in sediments of desert becomes clear, that in the period of fossilization of fauna (in the beginning of Meotian) warm and moist climate should have been prevailed on the territory of Kakheti, which assisted wide spreading of forest tracts.

Some years ago V. Trubikhin discovered rather rich site of vertebrate animals during paleomagnetic studies of represented here sediments in the Eastern Georgian (Kakheti) on the left bank of the river Iori, in the surroundings of Dzedzvtakhevi, which has a certain significance for the dating of Neogenic sediments of South Caucasus and clarification of issues of prohoresis of fauna. The site is barely dug. Only some searching works are carried out, but obtained bone material already gives possibility of characterization of fauna. By now the following are known in Dzedzvtakhevi fauna: *Gomphotherium* sp., *Crocuta (percrocuta) eximia, Felis attica, Dicerorhinus* sp., *Hipparion ex gr. elegans, Microstonyx cf. erymanthius, Gazella cf. deperdita, Tragocerus* sp., *Tragelaphinae gen., Karsimatherium aff. bazalethicum, Cervidae gen.* [Vekua, Trubikhin,1988]. Mentioned fauna and sediments containing bones are dated as Maeotian. Paleomagnetic investigation of bone-containing sediments confirms the same.

E. Kvavadze has performed palynological study of Meotian sediments of Dzedzvtakhevi and obtained interesting results. It turned out, that palynological spectrum is distinguished by diversity and abundance of pollen and spores in Dzedzvtakhevi sediments; trees and shrubs are well represented in spectrum, next to which spores of grassy plants are often found. Remains of tree-plants (58%) prevail in spectrum, among which Alnus 22%, Abies 11% dominate. Platanus 9% and Pinus 8% are represented with comparatively less number. From coniferous plants Picea 5%, Cedrus 1% are found. Remains of broad-leaved plants Fagas and Carpinus are nearly of the same quantity, and remains of Juglans, Tilia, Quercu and Salix are rare.

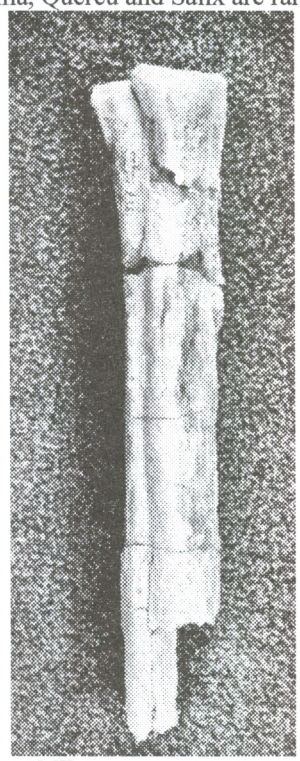

Fig.2. David Garedji. Giraffa sp. MT

Pollen of various grassy plants reaches about 23%, among which waterside and marshy forms (Spagnum) prevail. According to the data obtained E. Kvavadze concludes, that waterside wood would have been represented in the surroundings of Dzedzvtakhevi and neighboring territories in Meotian period, next to which perhaps spaces covered with comparatively open savannah-type light forest existed. Climate was mild, with warm winter and hot dry summer, similar that of Mediterranean.

Small, but stratigraphically interesting fauna of vertebrates, mentioned first by N. Vereshchagin [Veretshagin, 1959], was found in Kakheti, near village Japaridze.

Fauna comprises Proboscidae gen?, Hipparion sp, Rhinoerotidae gen?, Sus sp, Cervus sp; Giraffidae gen?, Gazella sp.

As it seen from the list, none of the forms is determined up to species because of fragmentariness of material, but it is clear, that we deal with Hipparion fauna and definition of its stratigraphic level is not difficult. L.Gabunia (1959) identified Hipparion of Japaridze with Hipparion of Gareji (*H. garedzicum*) and by it defined the age of Japaridze fauna as Meotian. We also consider Meotian age of Japaridze fauna to be correct.

Pliocene sediments are represented with rich, diverse fauna in Kakheti. Faunas of Kvabebi and Zemo Melaani are worthy of special note from Pliocene sites.

In some researcher's opinion, Pliocene begins with Pontus floor, which is represented with continental strata. Pontus fauna, as it is, is not found in Kakheti, but following stage is represented well.

Late Pliocene is represented with fauna, located in the sea sediments in South Caucasus. Bone-containing layers of known in literature site Kvabebi are dated by molluscous fauna as Middle Aghchagilian.

In faunistic complex of Kvabebi 21 representatives of six orders of mammalians are determined. Besides mammalians Struthio gigantea and Otistarda are found from the birds, and Testudo from the reptiles. By now, the following are defined in Kvabebi fauna: *Testudo cernovi, Struthio transcaucasicus, loriotis gabunia, Nyctereutes megamastoides, Canis* sp., *Ursus arvernensis, Therailurus* sp., *Lynx issiodorensis, Homotherium davitashvili, Hystrix cf. primigenia, Kvabebihyrax Kakheticus, Anancus arvernensis, Hipparion crusafonti, Dicerorhinus megarhinus, Propopotamochoerus provincialis, Euscladoceros* sp., *Pseudalces* sp., *Procapreolus* sp., *Protoryx heinrichi, Oryx* sp., *Gazella postmitilinii, Parastrepsiceros sokolovi, loribos aceros, Eosincerus ivericus* [Vekua, 1972].

It is evident that mainly elements of Roussillion fauna are represented in Kvabebi fauna. Homotherium, Anancus arvernensis, Dicerorhinus megarhinus, Hipparion and Propotamochoerus obviously indicate the close connection of Kvabebi and Roussillion. At the same time existence of deer (Eucladoserus, Procapreolus, Nyctereutes and some comparatively progressive forms) in Kvabebi fauna somehow makes it younger. Proceeding from mentioned, we [Gabunia, Vekua 1968] considered it possible to define Kvabebi fauna as faunistic complex of South Caucasus-Asia Minor, which crowns Neogenic history of development of Pliocene vertebrates fauna.

The analysis of Kvabebi fauna assures us that both European and Asian forms are represented here, but Asian elements considerably exceed. Namely, Asian forms are: *Nyctereutes megamastoides, Lynx issiodorensis, Propotamochoerus provincialis*, some hollow-horned. Abundance of mentioned Asian elements in Kvabebi makes us think that their core must be got into South Caucasus through Asia Minor. We should suppose that Caucasus mountains turned out to be insurmountable barrier for some animals and their migration to west was not accomplished. Even the fact, that diverse group of Hyracoidea did not penetrate to the west of South Caucasus, tells about it. Perhaps, the same barrier prevented penetration of hippopotamus to north at the beginning of Anthropogenic period.

Relation of vertebrate faunas of Africa and Europe in geological past is undoubted. It is clear, that basic core of savannah fauna of present-day Africa is related to Hipparion faunas of Europe and their migration should have been happened in second half of Neogene [Gabunia, Vekua 1968].

Discovery of Aghchagilian faunistic complex of Kvabebi greatly assisted clarification of problem of interrelation of Eurasian and African faunas. Namely, participation of Parastrepsiceros, Protoryx Eosyncerus, Kvabebihyrax, Struthio and other forms in Kvabebi fauna indicates that formation of savannah-type fauna of South Africa took place in the period of existence of Roussillion-Kvabebi fauna and at the expense of the migration of these faunas to Africa, perhaps, through so called Levantine passage.

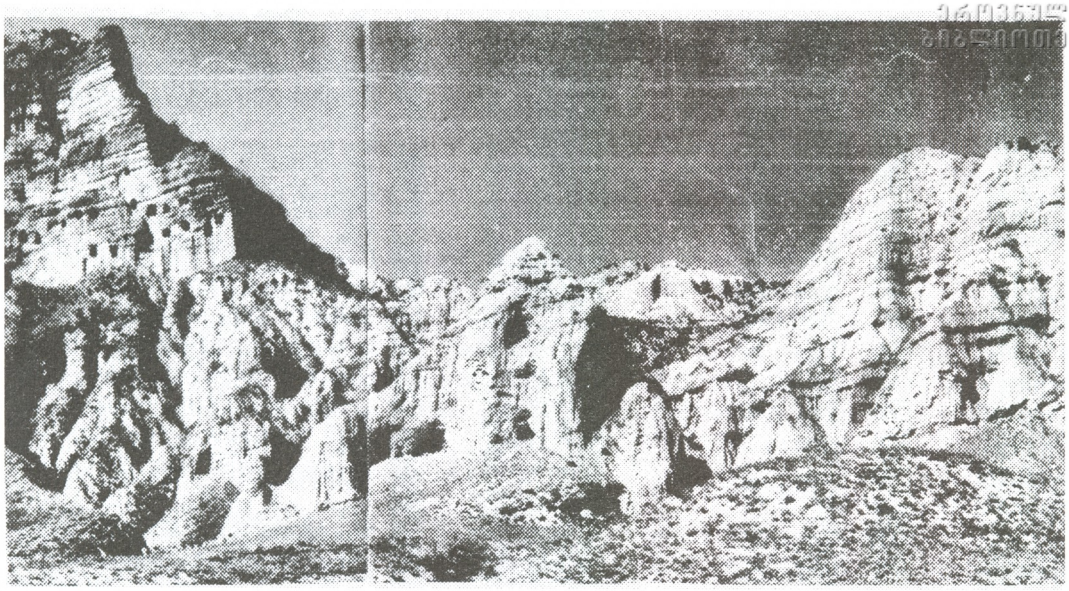

Fig.3. View on the Kvabebi site

Scientists thought, that there was no interrelation between Eurasian and African faunas nearly for the whole Oligocene. In their opinion, continental relation between Europe and Africa was set only at the beginning of Miocene, which conditioned migration of faunas from Eurasia to Africa and vice versa. It should be also noted, that migration of mammalians was more intensive from Eurasia to Africa, though a number of representatives of African savannah animals had already reached territory of Eurasia by the continental bridge at the beginning of Miocene. This relation becomes more intensive at the beginning of Pliocene and particularly in the second half of it, when there is the epoch of thrive of Roussillion-Kvabebi faunas. Abundance of preceding forms of African artiodactyls in Kvabebi fauna, such as Parastrepsiceros, Protoryx, Oryx, Eosincerus and others point to it. At the same time, the rate of prohoresis of these forms was so high, that by the end of Pliocene they had nearly settled the whole territory of Africa [Gabunia, Vekua 1968].

Prohoresis of faunas between Africa and Asia continues in Pleistocene; may be it is not so intensive as in Pliocene, but migration of diverse fauna of deer from Europe to Africa took place just at this period. It should be also noted, that this migration was nearly one-sided from Europe to Africa. We should also recollect, that existence of genus of giraffe in Gomareti fauna [Gabunia, Buachidze 1970] indicates the fact, that some representatives of mammalians were still able to reach Eurasia from African continent by the end of Pliocene.

Pliocene clay sands of continental origin are exposed on the territory of Eastern Georgia, at the village Melaani, in which fossilized bones of mammalians were found in small amount. As a result of studying this fossilized collection the following were defined in the composition of fauna: *Nyctereutes megamastoides* (basidigital bone), *Chasmaporthetes lunensis* (lower jaw), *Dinofelis abeli* (skull), *Anancus arvernensis* (canine), *Dicerorhinus megarhinus* (basidigital and carpal bones), *Sus cf. strozii* (lower jaw, fragment of skull, shoulder bone), *Cervus* sp. (bones of extremities), *Leptobos* sp. (lower jaw, teeth, bones of extremities).

Geological age of Zemo Melaani fossil fauna is not quite clear. According to general picture of geological structure of region we conclude that bone-containing layers should be of Pliocene age. It is clear that discovered fauna of mammalians is of great significance for solving this question. From the list of given fauna it becomes clear, that fauna is represented with forms,

characteristic to Lower-Middle Villafranche of Mediterranean [Azzaroli et al., 1988; Agusti, Moja-Sola, 1992]. The same is confirmed by the presence of Anancus arvernensis, Dinofelis, Leptobos and other Pliocene forms in fauna.

And finally, Zemo Melaani fauna is obviously close to faunistic complex of Kvabebi, which assures us, that fauna of Zemo Melaani and sediments containing it are of Aghchagilian age, but in comparison to Kvabebi it must be younger. Hence, it would be right to date these sediments as Late Aghchagil.

Zemo Melaani fauna, as well as Kvabebi fauna, inhabited perhaps in the conditions of relatively moist climate, which assisted the spread of forest and quite vast, marshy here and there, forest-steppe landscapes.

By the beginning of Anthropogenic period (Apsheron) change of physical-geographical conditions is obviously noticeable on the territory of Georgia: relatively warm and moderately hot climate, xerophytic vegetation is widely spread, and essential changes in vertebrate fauna composition should have been followed it. Earlier widely spread Hipparion gradually leaves its inhabitancy and Equus caballus occupies its place, and Mastodons are replaced by Elephantidae. Qualitatively new faunistic complex is formed, which started development of Quaternary mammalian fauna.

Rich paleontological material enables us to follow history of formation of Anthropogenic period fauna in Eastern Georgia, namely on the territory of Kakheti.

Transitional from Pliocene to Pleistocene period is well-represented in Kotsakhuri fauna. Against the background of scarcity of fossil vertebrate fauna, discovery of Kotsakhuri site on the territory of South Caucasus (Dedoplis Tskaro District) is undoubtedly important event. Some years ago, during the field expedition on south slope of Kotsakhuri ridge we discovered fossil animals, mainly bones of mammalians. Obtained material formed quite important faunistic collection.

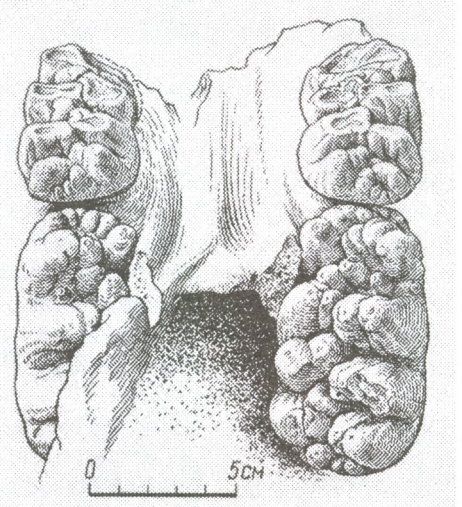

Fig.4. Anancus arvernensis (Cr. Et Job.) Pd^4, M^1, occlusal view

Rather strong strata of Aghchagil-Apsheronian sediments are exposed in the surroundings of Kostakhuri ridge. Detail description of its section is given by Trubikhin and Chepaliga.

Conglomerates of sandstones and pelitic clays are represented in the upper part of sediments of Kotsakhuri ridge in turn. Shells of molluscous fauna of fresh water are often found in this part, according to which it is easy to ascribe mentioned sediments to Apsheronian floor.

A.Chepaliga found shells of large size Unionids in the lowest part of Apsheron sediments, horizon of which is dated as Damashkin horizon. Damashkin horizon is generally characteristic for the lower part of Apsheronian sediments.

Lower part of section of Kotsakhuri ridge sediments is characterized by Agchagil fauna and dated well by sea mollusca.

Lower part of Kotsakhuri ridge sediments is magnetized positively, so it belongs to Epoch of Gaus, upper part is magnitized negatively and obviously belongs to Matu.yama. In the upper part of negatively magnetized Apsheron sediments Trubikhin separates horizon of little power, which is characterized with positive magnetism, and considers it as Jaramilo episode.

Geological age of complex of Kotsakhuri vertebrate fauna, which lies under sediments of Damashkin horizon, is disputable.

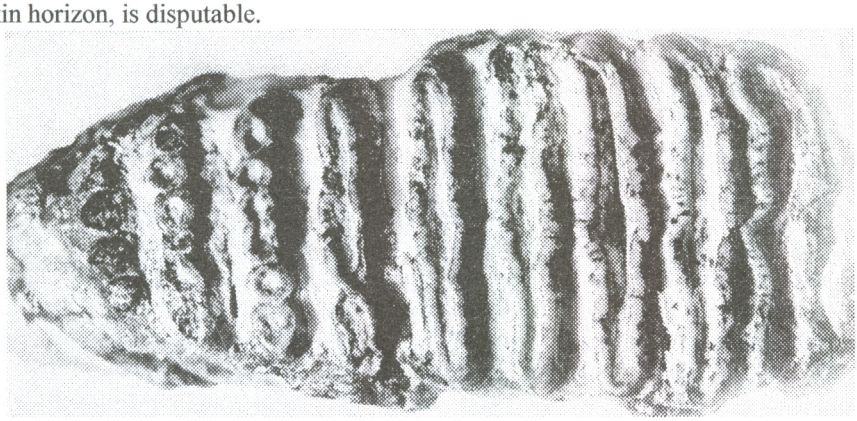

Fig. 5. Taribana. Archidiskodon meridionalis taribanensis Gabunia, Vekua. M^3 occlusal view.

V.Trubikhin considers these sediments and correspondingly fauna as Lower Apsheronian, with which we can not agree.

By now in Kotsakhuri fauna following are defined: *Archidiskodon meridionalis, Equus stenonis, Dicerorhinus etruscus, Megalocerus giganteus, Gazella* sp., *Protoryx* sp., *Leptobos* sp., *Testudo greaca, Struthio gigantea*. For defining the geological age of given fauna first of all representatives of Proboscidiforms, Equidae and Bovidae are worthy of attention. In the case of Kotsakhuri fauna our main attention is focused on Proboscidiforms. Evidently, Kotsakhuri Archidiskodon is the same, as *Arch. meridionalis taribanensis*, which is singled out as subspecies of taribanensis of south elephant [Gabunia, Vekua 1963]. Identity of Taribana and Kostakhuri elephants is undoubted: actually, their remains are found in analogous sediments and their sites are located side by side.

To define geological age of Kostakhuri fauna we will use *Arch. meridionalis taribanensis* and geology of its fossilization place.

According to D. Buleishvili's (1960) data Aghchagilian sediments of Kakheti territory are represented by two facieses: sea sediments, dated well with molluscous fauna, are located in the lower part, and in the upper part sea sediments gradually, without visible defect, passes into continental, which contain upper part of Agchagil and whole Apsheronian as well. Definition of boundary between Agchagil and Apsheron can not be done. It is only known, that sea Aghchagil is positively magnitized, and Apsheron – negatively.

Arch. meridionalis taribanensis is found in conditionally transitional sediments, represented by solid fine-grained sands.

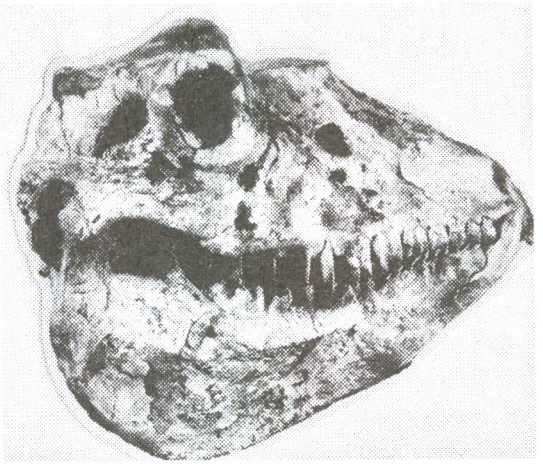

Fig.6. *Kvabebihyrax kachethicus* Gabunia, Vekua. Skull and mandible

It is known that systematisation of elephants is mainly based on morphological features of teeth, among which the number of tooth plates, number of plates at the distance of 10 cm. and thickness of enamel are important. Systematisation of southern elephant is exactly defined by these marks. The elephant, found in Valdarno, Italy, is considered as archaic, which has more plates on his molar (M3), than *Arch. meridionalis taribanensis*. It also differs by the little thickness of enamel and frequency of plates at the distance of 10 cm. By mentioned features *Arch. meridionalis taribanensis* differs from Khapri elephant, and undoubtedly is more archaic, than typical elephant of Valdarno and Khapri Archidiskon. Archaic morphological features of *Arch. meridionalis taribanensis* completely corresponds to its geological age – upper Aghchagil. Thus it is clear, that by now *Arch. meridionalis taribanensis* is elder than found in the Central and Eastern Europe southern archaic elephant.

Let us return to geological age of Kostakhuri fauna. We have seen that sediments containing remains of *Arch. meridionalis taribanensis* are of upper Aghchagil. At the same time Kotsakhuri fauna is also found in the analogous sediments. Besides, *Arch. meridionalis taribanensis* is also given in Kotsakhuri fauna. Hence, we can logically conclude that Kotsakhuri fauna is completely of Upper Aghchagil and corresponds to 17 biozone of Mein (MN 17).

E. Kvavadze carried out palynological investigation of rocks containing Kotsakhuri fauna and, in hes opinion, palynological spectrum of Kotsakhuri is characterized with an equal quantity of plant pollen. From tree-plants Platanus and Alnus are abundant. From coniferous Picea and Cupressus are met. Remains of pollen of Fagus, Carpinus, Querqus, Juglans and Tilia are comparatively scanty. From grassy vegetation, remains of Chenopodiacea and Artemisia are often found.

Special abundance of pollen of Alnus in samples indicates the wide spread of waterside forest on this sector, but steppe landscape is also widely represented next to forest. Climate should have been dry, continental in the surroundings of Kotsakhuri [Vekua, Kvavadze, 1981; Kvavadze, Vekua, 1993].

If we sum up palynological studies of Pliocene sediments of Iori plateau, we will find out, that in the period of spread of Pliocene sediments, mainly savannah-type Xerophytic landscape was presented on Iori plateau, here and there covered with waterside forest and marshy sectors. Climate should have been that of Mediterranean type, with mild moist winter and hot and dry summer [Vekua, Kvavadze, 1981].

There is lack of remains of vertebrate in main part of Pleistocene sediments on the territory of Kakheti. Therefore, if we find one or two isolated peaces on this territory, it is very important for stratigraphy. Quaternary sediments are widely presented in the north part of Shiraki, in the surroundings of Alazani valley, which overlie Upper Pliocene sediments of Alazani series (Pliocene, continental analogue of Aghchagil-Apsheron) incompatibly. Pleistocene sediments are represented with alternation of weakly dislocated, yellowish-reddish sands and grey clays here. Geological section of this territory is given by Jajanidze and Mamatsashvili (1968). As authors describe, alternation of yellowish-reddish sands, cemented clays and cemented cobble-stones overlie sediments of Alazani series by angular incompatibility in the surroundings of Kvemo Kedi. Mentioned investigators draw the lower border of Quaternary (Pleistocene) under the yellowish-reddish sands and consider them as sediments of Baku floor. Geologists made such conclusion on the basis of location of sediments on Alazani series and paleontological remains found there.

Fig.7. *Kvabebihyrax kachethicus*

Local population uses yellowish-reddish sands as a building material. They have noticed for a long time, that quartz sands of reddish color contain fossil bones. It is clear that great part of fossil bones, found here, have been lost because of carelessness, but a little part has got into the Institute of Paleobiology. According to paleontological studies Kvemo Kedi fauna comprises *Archidiskodon trogontherii, Megaloceros giganteus, Cervus elaphus*.

Finding together of *Arch. trogontherii* and *Megaloceros giganteus* in the sediments of Kvemo Kedi clearly points that mentioned fauna and sediments, containing bones, should be ascribed to the upper part of Lower Pleistocene or the beginning of Middle Pleistocene [Vekua, Khukhia, 1972].

Middle Pleistocene is represented by vertebrate fauna on the territory of Georgia, which is mainly connected with the activity of Stone Age man. In Western Georgian cave dwellings are often, where stone tools of Acheulean or Mustier cultures with accompanying remains of fauna in the form of left-over bones are defined. But unfortunately, such dwellings and sites of this period are comparatively rare on the territory of Eastern Georgia. From Stone Age dwellings we can name

Tsopi [Grigolia, Vekua, 1963], and from sites of fauna – Kvemo Kedi, Algeti (village Kesalo). One-toed *Equus caballus* dominates in the fauna of mammalians, characteristic for Middle Pleistocene. *Dicerorhinus mercki, Archidiskodon trogontherii, Ovis ophion, Gazella, Bos primigenius, Megaloceros giganteus* and others are rather frequent. It must be noted, that European elements dominate mainly in this fauna.

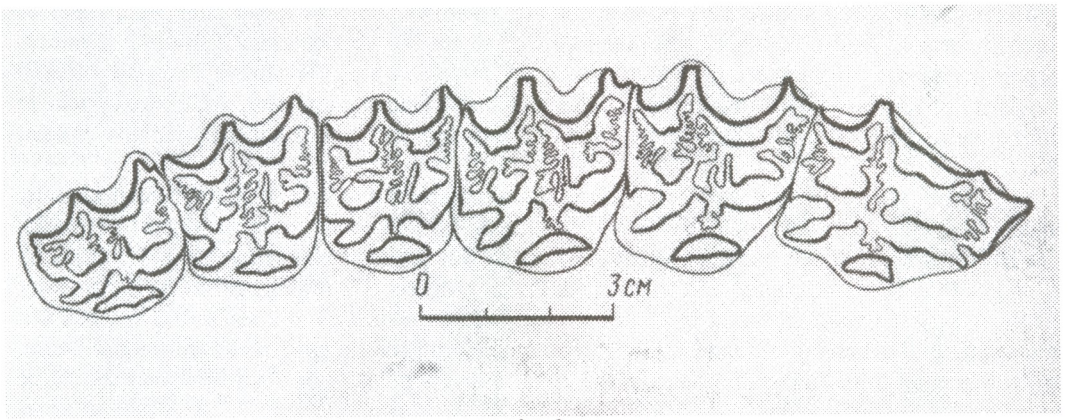

Fig.8. Kvabebi Hipparion crusafonti Vill. P^2-M^3occlusal View.

Mammalian fauna of Upper Pleistocene on the territory of Kakheti and generally in Eastern Georgia is almost the same as that of Middle Pleistocene, but considerably impoverished. Only the role of Sus scrota rises in fauna.

Faunistic complex of Holocene is almost similar of contemporary fauna, but relics of Pleistocene fauna are left here and there.

Physical-geographic picture changes in the direction of xerophytization on the territory of Eastern Georgia, in Kakheti, in Holocene.

Sectors covered with forest diminish, but steppe and half-steppe landscapes increase. Fauna of vertebrate correspondingly changes, in which Hyaena hyaena, Equus hemionus, Gazella, Camelus and others appear. In Bendukidze's opinion (1971), arid-loving endemic forms and thermophilic elements from Near East – Erinaceus, Meriones, Capra aegagrus, Ovis ophion and others become more and more spread in Eastern Georgia.

Fauna of vertebrate considerably lessened in the second half of Holocene because of influence of a man. Equus hemionus, Gazella, Ovis ophion became extinct finally. At the verge of extinction are Panthera pardus, Hyaena hyaena, Felis silvestris and others. Environment changed greatly. Forest tracts lessened substantially [Janelidze, 1971] and environment assumed the present-day form.

Conclusion

Some issues of history of Neogenic fauna are not perfectly elucidated to date, in spite of the fact that sites of Mio-Pliocene vertebrates are often found in Eastern Georgia. Namely, we know nearly nothing about initial stage (Middle Sarmatian) of development of Hipparion fauna. Middle Sarmatian is represented in South Caucasus with sea facies and remains of land fauna are found nowhere. Although, fauna of Upper Sarmatian is well presented, especially with the equivalent of Meotian, is represented by the faunistic complexes of Eldar, David Gareja and Dzedzvtakhevi, which enables us to reconstruct the history of fauna and paleogeographic picture of

Meotian period. Study of mentioned fauna is of great importance not only for comparison of biostratigraphic and continental layers, but also for clarification of theoretical questions of regularity of development of organic world.

Excavations carried out during last years and material obtained considerably broadened our knowledge on history of fauna of South Caucasus and particularly of Kakheti territory. Special significance is attached to discovery of Kvabebi site, where elements of Asian fauna and, which is more important, forms, characteristic for African fauna, is concentrated (Struthio gigantea, Propotamochoerus, Homotherium, Parastrepsiceros, Hippotraginus, Kvabebihyrax and others). Peculiarity of Kvabebi fauna enabled us to single out a new faunistic complex, which we oppose to Cimmerian together with Aghchagil in the form of lower floor of Upper Pliocene [Gabunia, Vekua, 1968].

South Caucasus was some kind of refugium, where warm and relatively moist climate, mosaic landscape and favorable living conditions of Mio-Pliocene were retained. Therefore, Giraffe-Paleotragus, which had been already extinct by this period, remained in South Caucasus to the end of Pliocene. Anthropoid ape, which is the latest (Meotis, 7-8 million years) on the territory of former Soviet Union, is found in Georgia, Gareji desert. Primates of Pleistocene age had not been found until discovery of remains of macaque in Kudaro (Terjola District) [Veretshagin, Lubin, 1960]. Macaque from Kudaro is found in the cultural layers of Acheulean-Mustier and should be considered as Neogenic relict.

Ursus spelaeus, nearly the companion of Old Stone Age man, had been almost extinct in Central and East Europe by the end of Pleistocene. But in the Western Georgia, on the territory of Abkhazeti, this predator was widely presented in the fauna of Mesolite [Burchak-Abramovich, 1960].

And finally, existence of certain refugium in South Caucasus assisted colonization and settlement of Early Hominids, which is attested by the site remains of Dmanisi hominids [Gabunia, Vekua 1993; Gabunia, et al., 2000].

Second half of Pleistocene is represented with relatively poor and monotonous fauna. It is obvious, that the role of Asian element in fauna of South Caucasus greatly diminished from the beginning of Pleistocene, accordingly the influence of European forms increased. Present-day fauna of mammalians had already developed in the Pleistocene in South Caucasus, which became noticeably impoverished under the influence of a man in Holocene.

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კახეთისა და მიმდებარე რაიონების ნეოგენურ ხერხემლიანთა მრავალფეროვნების დინამიკა

ვეკუა ა., ლორთქიფანიძე დ.

საქართველოს ეროვნული მუზეუმი

(მიღებულია 23. 05. 2011)

რეზიუმე

ნამარხ ხერხემლიანთა ფაუნას გადამწყვეტი როლი ეთმობა კონტინენტური ნალექების გეოლოგიური ასაკის დადგენისა და მომიჯნავე რეგიონების შესატყვისი ასაკის ნალექებთან შეპირისპირების საქმეში. აღმოსავლეთ საქართველოში მოპოვებული პალეონტოლოგიური მასალა (დავით გარეჯის, ქვაბების, კოწახურისა და სხვა ადგილსაპოვებლების ფაუნები) შესაძლებლობას გვაძლევს თვალი გავადევნოთ წარსულის პალეოგეოგრაფიული სურათის ევოლუციას და გამოვყოთ გარემოს ცვლილებების შესაბამისი ეტაპები.

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Plant Physiology

DETERMINATION OF THE CYTOTOXICITY OF CHITIN-BINDING MISTLETOE (*VISCUM ALBUM* L.) FRUIT LECTIN (MChbL) ON HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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Abstract

The in vitro effects of chitin-binding mistletoe lectin MChbL on human peripheral blood cells have been investigated. MChbL inhibited proliferation of PBL cells at concentration of 500 μg/ml and showed cytotoxic effects. Low concentrations of MChbL were less cytotoxic to peripheral blood cells and exhibit similar results as ConA at the concentration of 1μg/ml. Application of mistletoe lectin as natural biopesticide at dose-dependent manner is discussed.

Key words: Cytotoxicity; Mistletoe lectin; Peripheral blood lymphocytes; *Viscum album.*

Introduction

The insecticidal activity of plant lectins against a large array of insect species belong to the Coleoptera, Homoptera, Diptera and Lepidoptera order has been well documented [Coelho et al., 2007; Ohizumi et al., 2009]. Insecticidal activities were found to be associated mostly with the two main groups of plant lectins: monocot mannose-binding and chitin-binding lectin groups [Rudiger & Gabius, 2001]. Known as natural defense agents, plant lectins have been implicated as antibiosis factors against insects and considered as promising candidates for biological pesticides.

Mistletoe (*Viscum album*) fruit lectin (MChbL) belongs to the chitin-binding lectin group with sugar-binding specificity exclusively to β-1,4-GluNAc polimer [Keburia, Alexidze, 2001]. In the previous studies we demonstrated anti-nutritive effects of MChbL against *Apamea sordens* Hufn. and *Agrotis segetum* Schiff. (Lepidoptera: Noctuidae) larvae. MChbL exhibited proteinase inhibitory activity and affected larval development at different growth stages [Keburia et al., 2010].

Mistletoe is considered to be a toxic plant, and its content of toxic lectins lends support to this. Poison centers report toxicity of the whole plant, but especially the berries [Stirpe, 1983]. Mistletoe proteins, particularly, viscotoxin and some mistletoe lectins known to be toxic to mammals, on tumor cell lines in culture [Urech, 1995].

In the present paper we analyzed MChbL cytotoxicity towards human peripheral blood lymphocytes (PBL) and determine its possible mammalian toxicity.

Materials and Methods

Human peripheral blood lymphocyte (PBL) culture was used to study MChbL cytotoxicity by mitogen stimulated dimethylthiazol, diphenyltetrazolium bromide (MTT) assay. Separation of peripheral blood lymphocytes (PBL) was performed as follows: The peripheral blood of 10 healthy donors aged 20 -50 were studied. Each sample of whole blood was diluted 1:1 ratio with Ca^{2+} and Mg^{2+} free Hank's balanced salt solution (HBSS, Gibco) and 10 ml of this mixed blood was overlayered onto 3 ml Histopaque ($1.077g/cm^3$ density) solution (Sigma). After centrifugation at 770 g for 45 min at room temperature, the interphases of PBL was aspirated and cell suspension was washed twice in HBSS at 400 g for 10 min, re-suspended in 1 ml of medium RPMI 1640 (Sigma), counted in Haemacytometer and concentration was adjusted at $2X10^6$ ml with medium, supplemented with 10% fetal bovine serum (FBS, Sigma).100 µl of this suspension was added into wells of 96 well microplate in duplicates and each well was filled with 80 µl media supplemented with 20 µl mitogen (ConA, Sigma) or 20 µl MChbL. The wells without mitogen or MChbL were considered as blank (Bl) wells. Different dilutions of MChbL protein and mitogen were used. 20 µl of MTT solution (Sigma, 5 mg/ml PBS - phosphate buffer solution) was added into each well after 72 h incubation time at 37°C. During next 4 h incubation time the formazan crystals were produced. The media was removed from wells carefully and 100 µl solution of 10% SDS (sodium lauryl sulfate)/0.1m HCL was added. After 3h incubation at 37°C the crystals were dissolved and the optical densities of the wells were read using spectrophotometer Multiscan MCC at 570 nm wavelength.

Results and Discussion

The MTT assay is colorimetric assay for measuring the activity of enzymes that reduce yellow MTT to purple formazan crystals, in the metabolically active mitochondria of living cells. The main application allows assessing the viability and the proliferation of cells. It can also be used to determine cytotoxicity of potential different agents and toxic materials, since those agents would stimulate or inhibit cell viability and growth. The reduction of tetrazolium salts is now recognized as a safe, accurate alternative to radiometric testing [Wilson, 2000]. The formazan crystals are solubilized by the addition of a detergent. The color can then be quantified by spectrophotometric means, measuring at a certain wavelength (usually between 500 and 600 nm). The reduction takes place only when mitochondrial reductase enzymes are active and therefore conversion can be directly related to the number of live cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated cells, the effectiveness of the agent in causing death of the cells can be deduced. Increasing cell number result correlates with an increase amount of MTT formazan formed and an increase in absorbance [Wilson, Anne P., 2000]. The result of study is shown in Table 1. Any of doze of mitogen (Con A) have stimulated the human peripheral blood lymphocytes OD – 0.344 (100µg), 0.467 (10µg) and 0.198 (1µg), in compare to blank (B1) OD 0.173, whereas MChbL in both concentrations applied, inhibited the proliferation of PBL cells possibly decreasing the amount of the cells (OD 0.100 and OD 0.060 at concentrations of 10 µg/ml and 500 µg/ml respectively). Particularly, this was obvious at higher concentration of MChbL applied (OD 0.060). Apparently, MChbL negatively affects cell functioning and accordingly, reduces their number.

Some plant lectins expose toxic and pathological lesions on mammals. In vitro, plant lectins effect lymphocyte mitogenesis, aggregate immunoglobulin induce histamine release from basophils and mast cells. The *in vitro* administered mistletoe extracts and pure mistletoe lectins inhibited the growth of all tumor cell lines thus show the therapeutic effects [Jansen et. al., 1995].

MChbL expose cytotoxical effects to PBL when administrated at high concentration. However, low concentrations of MChbL were less cytotoxic to peripheral blood cells and exhibit similar results as ConA at the concentration of 1μg/ml. Most likely, approaches to use MChbL as natural biopesticide must be considered at dose-dependent manner and administrated in the nontoxic range.

Table 1. Proliferation of human peripheral blood cells by ConA and MChbL

Healthy donors	Bl (control)	Con A 100 μg/ml	Con A 10 μg/ml	Con A 1μg/ml	VAC 10 μg/ml	VAC 500 μg/ml
Donor N 1	0.154	0.362	0.424	0.15	0.103	0.072
Donor N 2	0.139	0.491	0.623	0.15	0.107	0.062
Donor N 3	0.15	0.237	0.379	0.155	0.106	0.080
Donor N 4	0.18	0.371	0.42	0.171	0.092	0.029
Donor N 5	0.19	0.232	0.328	0.186	0.100	0.078
Donor N 6	0.173	0.239	0.378	0.179	0.114	0.080
Donor N 7	0.18	0.403	0.562	0.260	0.108	0.048
Donor N 8	0.156	0.293	0.473	0.198	0.084	0.049
Donor N 9	0.216	0.444	0.587	0.297	0.106	0.059
Donor N 10	0.199	0.373	0.499	0.231	0.076	0.038
Mean of dates	**0.173**	**0.344**	**0.467**	**0.198**	**0.100**	**0.060**

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ფითრის (*Viscum album*) ქიტინ-დამაკავშირებელი ლექტინის (MChbL) ციტოტოქსიკურობის დადგენა ადამიანის სისხლის პერიფერიული ლიმფოციტების მიმართ

გაჩეჩილაძე ნ., ხურციძე ე., გაიდამაშვილი მ.

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(მიღებულია 11.10.2011)

რეზიუმე

გამოკვლეულია ფითრის ქიტინ-დამკავშირებელი ლექტინის MChbL *in vitro* გავლენა ადამიანის სისხლის პერიფერიულ ლიმფოციტებზე. MChbL თრგუნავდა ლიმფოციტების პროლიფერაციას ლექტინის 500მკგ/მლ კონცენტრაციის გამოყენების პირობებში. დაბალი კონცენტრაციებით MChbL ავლენდა სუსტ ტოქსიკურობას სისხლის პერიფერიული ლიმფოციტების მიმართ და ახდენდა ConA მსგავს ეფექტს. განიხილება ფითრის ლექტინის ბუნებრივ ბიოპესტიციდად გამოყენების შესაძლებლობა დოზასთან დამოკიდებულების პირობებში.

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Letter to Editor
Botany

FRUIT TREES OF SVANETI (NORTH-WESTERN GEORGIA)

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Abstract

The article demonstrates material on fruit tree diversity described during field expeditions in Svaneti. The list and distribution of aboriginal cultivars are presented. The role of folk selection works in development of local populations is described. The domestication of native wild progenitor species is discussed.

Key words: Domestication, fruits, polyploidy, *Prunus cerasifera*, Svaneti,

Climatic, soil and geographic conditions of Svaneti is very suitable for development of orchards. The zonal distribution of fruit trees was studied by N. Ketskhoveli. Zonation plays important role in origination of fruit varieties. Seed trees such as apple and pear are widely distributed in high-mountain zone of Svaneti [Ketskhoveli, 1957]. The study of fruit varieties in Upper Svaneti was carried out in 1975-1977 and in Lower Svaneti in 1978-1980 [Zhizhilashvili et al., 1977; 1980]. The list of fruit trees and information on their origin was collected in villages from local population.

The cultivated varieties were growing together with wild individuals in orchards. The wild forms were very polymorphic and represent original material for breeding [Ketskhoveli, 1957].

Svanetian population always selected wild fruits and planted in private plots. Afterwards, they selected the best individuals and generate cultivated forms of fruits. As a result cultivated varieties of fruit trees are distributed in Svaneti today.

The selective work of local peasants was directed on adaptive characters of cultivars to local climatic and soil conditions. Special efforts have been undertaken in mountain regions to create places for establishment of orchards of fruits and vineyards. For this purpose, they should cut forest and develop orchards of wild fruit trees. So far there are remained orchards of wild pear - *Pyrus caucasica*, where many individuals are planted together in place of former forest and are surrounded by fens. This process is the first step of cultivation of wild individuals.

Fruit trees in Svaneti are represented by seed and stone fruits and nuts.

Seed fruits belong to *Rosaceae* family, *Pomoidae* subfamily. The representatives are: Apple (*Malus*), pear (*Pyrus*), quince (*Cydonia*).

Apple is represented by commercial varieties from 600 to 1200 m a.s.l. Local varieties are distributed at higher elevations 1200 m to 1600 m a.s.l. and the highest distribution place is in v. Koruldashi (1950 m a.s.l.). Local varieties of apple – "Turashauli" and pear – "Khechechuri" are very rare and threatened with extinction.

Wild apple - *Malus orientalis* ("Mazhalo") in Georgia is characterized by high polymorphism. It is widely spread in forests of Svaneti. The rootstocks of wild apple are used for grafting of cultivars. Many local varieties are generated from the wild apple in Georgia.

In spite of the fact that the wild apple is widely spread in forests of Lower Svaneti, this region is pure by local cultivars of apple. Both in Upper and Lower Svaneti forest-orchards are occurred where wild apple is used as rootstock for cultivars.

The following local cultivars of apple are found in Lower Svaneti: "Antroli", "Turashauli", "Abilauri"; in Upper Svaneti – "Saadreo Vashli", "Kitra Vashli", "Chuberis Tsiteli", "Gazafkhulis Vardi", "Lushnu Visg" ("Svanuri Vashli"), "Tsitela", "Gverdtsitela".

"Antroli" is the local variety of apple found in forest as seedling and replanted in orchards. Some hundred year old trees have been found during expedition. The fruit is like to "Kitra Vashli" group. It is adapted to local climate and is disease resistant. Spring frosts do not injure this cultivar. Fruit is of medium size, skin is thick, rough, and fleshy part is very juicy, not aromatic. It is used for establishment of forest-orchards.

"Turashauli" apple distributed in Racha-Lechkhumi, Meskheti, Kartli, and Svaneti are similar by morphology. The difference concerns colour and sometimes taste. The varieties of this cultivar are generated from local wild populations and the forms are described by Khomizurashvili (1939). "Turashauli" is 10-12 m height, high-yielding, resistant to spring frosts, harvesting every second year. Fruit is of medium size, hard, with thick skin, greenish-yellow background with reddish stripes. Brown spots are spread under skin, fleshy and juicy, sweet and slightly acid.

"Abilauri" is distributed in Lower Svaneti in peasant private yards. Tree is big with round crown. Fruit is of medium size, flat, round; skin is thin, yellow or green, fleshy and juicy, sweet, slightly acid.

"Vashli N1" was found in Lentekhi distr., v. Naghomari in 1982. This individual was found as seedling by local peasant in forest and was replanted in the yard 30-40 years before description. Tree is big, fruit of medium size (5.2 x 4.3 cm), looks like "Kitra Vashli" and "Antroli". Fruit is green, fleshy and juicy, sweet, high yielding, resistant to disease.

Besides local varieties introduced cultivars are found in Svaneti: "Peasgood Reinette", "Canada Reinette", "Lechkhumuri Shafran", "Moore Shafran", "Pippin Shafran", "Bergamot Reinette", etc. Two cultivars – "Pippin Shafran" and "Bergamot Reinette" are distributed above 1500 m. Highest yield from introduced cultivars of apple have "Winter Golden Pearmain", "Canada Reinette", "Yellow Bellflower".

Pyrus distributed in Svaneti belongs to ancient fruit trees of this region. Pear is widely distributed in R. Tskhenistskali gorge up to 1200 m a.s.l. The amount of pear decreases at higher elevations and reaches 1600 m with some individuals in a village.

Wild pear (*P. caucasica*) is very polymorphic species. The local varieties of pear are generated by populations of this wild species during century-old selective works of peasants. The originated local cultivars belong to "Panta Mskhali", "Kalos Mskhali" and "Khechechuri" groups [Khomizurashvili, 1939]. The young trees are used as rootstocks for cultivars. "Gulabi" group of cultivars is characterized by high quality of fruits. The origin of "Gulabi" group is unknown.

Most local varieties of pear in Svaneti are ripened in autumn. Most of them are very juicy and have dark brown middle part. Therefore the quality of local varieties is low for commercial point of view. The exception is "Khechechuri", which has no dark middle fleshy part. The introduced pear cultivars in Svaneti are: "William's", "Beurrè Ardanpon", "Beurrè Bosc", "Saint Germain", "Beurrè d'Anjou", "Forest Beauty".

The local varieties of pear are widespread in Svaneti. The most widely are distributed: "Shavi Mskhali", "Katsistava", "Lechkhumuri Gulabi", "Khechechuri", "Milakhuri", "Samariobo", "Saselo" and "Saenkenebo". Summer varieties are: "Shavi Saselo", "Tetri Saselo", "Lechkhumuri Gulabi", "Gombro", "Kapistona", etc. Winter varieties are: "Krua Mskhali", "Zamtris Khechechuri", "Magara". Group of "Panta Mskhlebi" (*P. caucasica* group) is represented by "Kviristava", "Samariobo", "Milakhuri", "Chikharuli", etc. The most widespread in Svaneti are

autumn pears with large fruits: "Shavi Mskhali", "Katsistava", "Saenkenebo", characterized by high quality of fruits.

Quince (*Cydinia oblonga*) is represented in Svaneti by both varieties characterized for Georgia: apple like and pear like. The number of individuals is few.

Plum genus (*Prunus*) is represented by several species: Plum (*Prunus domestica*), Tkemali (*P. cerasifera*), Sweet Cherry (*Cerasus avium*), Cherry (*Cerasus vulgaris*).

Plum is not widely spread in Svaneti, but some varieties occur: 'Tskalkliava", "Tetri Kliavi", "Otori", "Chanchuri", etc. The last variety is most common especially in Lower Svaneti. Both, cultivars and wild Tkemali are the most widespread fruit trees in Svaneti. The varieties differ by size of fruits. Some of them are trees, others shrubs. There are thorny and non-thorny varieties, with small and large leaves. The fruit form might be round, oblong, compressed, acuminate, etc., difference in fruit colour: red, yellow, dark red, white, rose. Taste might be sour, sweet, sourish-sweet. Fruit is fleshy and juicy, or dry. The form of stone might be round or compressed. Flesh part might be separated from stone or not. Commonly in most varieties of Svaneti stone is easily separated from the flesh part of the fruit. This character is not common for Tkemali varieties in general. The diploid forms of *P. cerasifera* are polymorphic but some polyploid forms are found in Svaneti [Baiashvili, 1983].

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სვანეთის ხეხილოვანი კულტურები

ბაიაშვილი ე.

ილიას სახელმწიფო უნივერსიტეტის ბოტანიკის ინსტიტუტი

(მიღებულია 23.05.2011)

რეზიუმე

ნაშრომში წარმოდგენილია სვანეთში საველე ექსპედიციების დროს აღწერილი ხეხილოვანი კულტურების მრავალფეროვნება. მოცემულია აბორიგენული ჯიშების ჩამონათვალი და მათი გავრცელება ადგილმდებარეობის მიხედვით. ხალხური სელექციის როლი ადგილობრივი ჯიშების და ფორმების ჩამოყალიბებაში და შექმნაში. განხილულია ადგილობრივი ველური წინაპარი სახეობების კულტურაში შემოსვლის პროცესი.

ინსტრუქცია ავტორთათვის

სამეცნიერო ნაშრომი გამოიცემა ინგლისურ ენაზე, მას უნდა დაერთოს რეზიუმე ინგლისურ და ქართულ ენაზე, სამეცნიერო მიმართულება, სათაური, ავტორთა გვარები და მათი სამუშაო დაწესებულების დასახელება, საკვანძო სიტყვათა მოკლე (4-6) სია.

წერილის მოცულობა არ უნდა იყოს 5 გვერდზე ნაკლები და 12 გვერდზე მეტი. წერილი უნდა გაფორმდეს შემდეგი რუბრიკაციით: შესავალი და მიზნები (Introduction), მასალა და მეთოდები (Materials and Methods), შედეგები და მათი განხილვა (Results and Discussion), დამოწმებული ლიტერატურა. უკანასკნელი უნდა იყოს დალაგებული ანბანის მიხედვით, ხოლო ტექსტში წყაროების მითითება უნდა ხდებოდეს ფრჩხილებში ჩასმული ავტორის გვარითა და წლით [Lernmark, Hagglof 1981].

მითითებული ლიტერატურა წარმოდგენილი უნდა იყოს შემდეგნაირად:

ჟურნალის შემთხვევაში

Carvalho C., Pereira H., Pina C. *Chromosomal G-dark bands determine the spatial organization of centromeric heterochromatin in nucleus.* Mol. Biol. Cell, **12**, 5, 3563-3572, 2001.

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Kuhn T.S. *The structure of scientific revolutions.* Chicago, IL, Chicago Press, 2000.

Brush S. *Flowing waters or teeming crowds*. In: Mental Models. D. Gentner (Ed.), Chicago IL., Chicago Press, 865-900, 2001.

მასალა რედაქციაში წარმოდგენილი უნდა იყოს ქაღალდზე ამობეჭდილი და დისკეტით (ან CD-ით). წერილი ერთი ფაილით უნდა იყოს შენახული (ცალკე ფაილად შეიძლება ილუსტრაციების წარმოდგენა), ხოლო ფაილის სახელწოდება წერილის პირველი ავტორის გვარს უნდა ატარებდეს.

ქართული ტექსტისთვის ოპტიმალური ფონტებია AcadNusx და AcadMtavr, ინგლისური ტექსტებისთვის - Times New Roman. შრიფტის ზომა - 12 პუნქტი, ინტერვალი - 1,5. ცხრილებში დასაშვებია უფრო მცირე ზომის შრიფტები. წერილი უნდა დაიბეჭდოს A4 ფორმატით, ზევით და ქვევით - 2,5 სმ., მარცხნივ – 3 სმ. და მარჯვნივ – 2სმ. დაშორებით. ცხრილები, გრაფიკები და დიაგრამები (მხოლოდ შავ-თეთრი) შესაძლებელია დამზადდეს როგორც Microsoft Word-ში, ისე Excel-ში, ფოტოსურათები მიიღება აგრეთვე ორიგინალების (არაელექტრონული) სახითაც.

ჟურნალის გამოცემა ავტორთა ხარჯებით ხორციელდება. თანხა რედაქციაში უნდა შემოვიდეს ნაშრომზე დადებითი რეცენზიის მიღებისთანავე. ნაშრომის რეცენზირება ანონიმურია და ავტორს აქვს უფლება მიიღოს ან არ მიიღოს რეცენზენტის შენიშვნები. უკანასკნელ შემთხვევაში ნაშრომი, დამატებით გაეგზავნება სარედაქციო საბჭოს ერთ-ერთ წევრს. მეორე უარყოფითი დასკვნის შემთხვევაში, ნაშრომი არ გამოქვეყნდება.

ნაშრომის ჩაბარება შეიძლება სამუშაო დღეებში, 12-დან 16 საათამდე, შემდეგ მისამართზე: თბილისი, რუსთაველის გამზირი 52, საქართველოს მეცნიერებათა აკადემია, ბიოლოგიის განყოფილება, IV სართული, 429 ოთახი, ტელ: 293-58-92, პასუხისმგებელი მდივანი – მაია გრიგოლავა.