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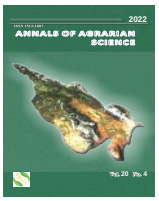

Improvement of Unique Economic Indicators in Georgian Breeds of Mulberry Silkworm

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A B S T R A C T

Methods of improving the viability of Georgian breeds of silkworm (Digomi 1 and Digomi 2) with excellent properties are considered in the work. The cocoons of the breeds of the initial Digmuri group are characterized by a long thread (2000 m) and high silkiness (24-25%). The only disadvantage of these breeds is that they have a relatively low viability, and this work was carried out just to improve this deficiency. As a result of carrying out complex selection work to increase the viability of the initial breeds, new improved hybrid combinations were obtained, which in their biotechnological properties are equal or higher than the initial breeds.

Key words: *mulberry silkworm, selection, cocoon, viability.*

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Introduction

In the Georgian gene pool of the mulberry silkworm, breeds bred by both old folk selection and scientific selection are preserved. Breeds bred by folk selection are characterized by a short thread and a cocoon with a thin shell. At the beginning, the breeds bred by scientific selection were also characterized by a short thread (700-900 m and did not even differ in purity, metric number 2000-2200), as a result of which it was necessary winding of 6-7-cocoons together to obtain raw silk. In subsequent years, the biotechnological parameters of breeds used in selection gradually improved and the length of the thread of breeds bred in 1985-1990 (Digomi 1 and Digomi 2) reached 1800-2000 m, and the metric number was more than 3500. It's true, these breeds are old enough, but they are characterized by high biotechnological parameters, due to which their use in selection works (in the form of a starting component) and in hybrid combinations (for production) gives a very good result, so it is important not only to preserve them, but also to improve their viability, in view of the fact that they are characterized by low viability, so selection was aimed at improving their viability.

Material and procedure

In the experiment, the local breeds Digomi 1 and Digomi 2 were taken for improvement, and the breeds with high viability also of local origin: Tbilisuri and Kartli were selected as an improving component [2]. The main leading features for the selection were the length of the cocoon thread of at least 1800 m, the purity of 3500 metric number heterogeneity inside the cocoon - no more than 30, between cocoons-17 and total -35, the caliber of the cocoon is -16-19 mm [3.4].

In sericulture, the effectiveness of selection work depends on complex factors, such as: selection of a qualitative initial material, the level of inheritance of selected selection features, the presence of genotypic correlation between them and the intensity of

selection. A complex of methodological questions that includes a whole cycle of the selection process includes: selection of parental forms, respectively, with the intended task, obtaining the initial population and reproduction of the selection material.

To improve viability, the moths, which emerged on the first day, the eggs, which were layed on the first day and larvae which appeared on the first day were used. Depending on how viable the pupa is, the process of metamorphosis proceeds so normally and quickly, so just such individuals were selected and left. The selection of moths was also carried out according to their viability, the eggs of such moth which survived for another 6-7 days after the laying the eggs were selected for reproduction. Blood infusion method was also used as well, and related breeds with high viability were selected as enhancers.

The method of preliminary prediction-determination of proteins in hemolymph was used for evaluation of initial and improved breeds, [5,6].

Results and analysis

Breeds for improving Digomi 1 and Digomi 2 have the following biotechnological indicators: silkiness of the alive cocoon 24.0-25.0%, silkiness of the air-dry cocoons 49.5-50.0%, raw silk yield 43.0-43.6%, silk filament length 1900-2000 m, dry cocoon yield ratio 22.1 (Table 1). As noted above, these breeds are characterized by high biotechnological properties and only need to improve their viability. To this end, the moths emerged after feeding of mulberry silkworm were crossed with the moths of the breeds- improvers Tbilisuri and Kartli, which are characterized by high viability. As a result, F_1 generation was obtained, the selected healthy eggs (grain) of which was revived artificially, after their feeding the biotechnological indicators of the hybrid generation F_1 were slightly better than of the previous, initial generation.

Table 1. *Biotechnological indicators of the initial material*

Characteristics	Breeds	Basic statistical parameters					
		∑	n	M	δ	m	C
Raw mass of alive cocoon g	Digomi 1	36.55	18	2.03	0.189	0.044	9.30
	Digomi 2	56.45	26	2.17	0.203	0.040	9.33
Cocoon shell weight, mg	Digomi 1	450	540	3749.3	18	430.52	157.57
	Digomi 2	470.3	26	468.09	139.338	27.326	29.77
Silkiness of an alive cocoon	Digomi 1	436.5	20	21.83	6.880	1.538	31.52
	Digomi 2	444.3	2	20.36	8.580	2.477	42.14
Length of cocoon thread, m	Digomi 1	34514.3	20	1725.72	612.693	137.002	35.50
	Digomi 2	32244.3	19	1697.07	611.992	140.401	36.06
Metric number of cocoon	Digomi 1	62989.3	21	2999.49	1014.349	221.349	33.82
	Digomi 2	65927.3	21	3139.40	1062.354	231.825	33.84

At a subsequent stage, work was carried out by analytical selection and attention was intensified again to improve viability (along with preserving other indicators) and fixation in generations. As a result of selecting the best individuals and crossing with the initial forms, complex hybrid combinations were obtained, i.e., grain of the second F_2 generation, in these breeds, work on preserving technological indicators and improving viability was again continued.

Table 2. Results of three-day feeding of mulberry silkworm (average)

N		Digomi 1	Digomi 1_2	Digomi 2	Digomi 2_2
1	Amount of grains in 1 g, pcs	1600	1595	1600	1610
2	Number of larvae in 1 g, pcs	2220	2240	2240	2250
3	Grains revitalization, %	96.7	98.7	96.7	98.4
4	Duration of feeding, days	30	30	30	30
5	Silkworm viability, %	88.0	91.4	88.6	92.5

6	**Mass of living cocoon, g.**	2.12	2.2	2.1	2.2
7	**Shell weight, mg.**	510	554	514	570
8	**Silkness fresh cocoon, %**	24.1	26.4	24.5	25.9
9	**Cocoon crop with 1 g larvae, kg.**	4.1	4.5	4.2	4.6
10	**Among them: normal cocoons, %**	94.8	95.6	93.9	95.5
11	**Double cocoons, %**	2.2	2.0	3.0	2.2
12	**Epmty, %**	3.0	2.4	3.1	2.3
13	**Cocoon winding capacity, %**	90.6	91.2	91.5	92,7
14	**Average thread length**	1990	2100	2000	2170

After feeding the larvae of the mulberry silkworm and emerging the moth, the above-mentioned crossing method was repeated, as a result of which grain of F_3-generation was obtained, the biotechnological indicators of which are much better than those of the original breeds.

Average biotechnological values obtained as a result of three-day feeding of mulberry silkworm of Digmuri group breeds are given in Table 2.

Discussion of the results

As noted above, the only disadvantage of Digmuri breeds is that they have relatively low viability under extreme conditions and just in order to improve this deficiency, this work was carried out. Selection work for the improving the marked breeds was carried out with strict selection and input of blood, on the basis of which families and individuals with high biotechnological indicators were selected, on the basis of which in the future it is possible to create new breeds with higher biotechnological performance and with much higher viability. [10,11.12].

From the data of Table 2 it can be seen that in terms of the average biotechnological indicators of three breeds, promising experimental breeds exceed the initial breeds and mainly meet the regulatory requirements of the breeds established for breeding.

The best indicators are: yields of alive cocoons with 1 g of grain, which is 4.5-4.6 kg and 0.4 kg more than of the original breed. As for the main goal of our experiment- to increase viability, this indicator increased from 88.0-88.6% in the initial breeds to 91.4-92.5%, which is a great achievement in the selection work of mulberry silkworm.

As can be seen from the table, as a result of selection work, it became possible not only to preserve the main features during selection (thread length and purity) in the marked breeds (Digomi 1 and Digomi 2), but also their significant improvement.

At the same time, in selected for reproduction individuals of experimental breeds, the average length of the cocoon thread increased by 110-170 m.

In the breeds of the Digmuri group, such families were identified, the length of the cocoon thread of which exceeded 2100 m, and in some individuals this figure reached 2400 m. Due to the large length and purity of the thread from the cocoons of the Digmuri breeds, it is possible to obtain raw silk that is used in the production of thin and expensive silk fabric.

As for the other biotechnological indicators, they have improved in many ways in experimental breeds [12]. Together with that, according to the main characteristics of selection (length of cocoon thread, purity and improvement of viability), we justify our preliminary goal. Despite this, work to improve and stabilize these features will be continued again, due to the large potential of experimental breeds.

As noted above, the method of preliminary prediction - the method of determining proteins in hemolymph - was used to evaluate the initial and evolved breeds. As you know, there is a direct correlation between the mass of silk glands and the silkiness of the cocoon - the greater the mass of silk glands, the higher the silkiness of the cocoon. The mass of silk glands grows especially intensely from the third day of the fifth age to the eighth day, i.e. till the forming of cocoon , due to the nutrition of mulberry caterpillars rich in carbohydrates, proteins and nitrogen. Approximately 70% of the mass of silky glands is produced as a result of the processing of proteins obtained from mulberry leaves, and the remaining 30% by the synthesis of hemolymph of mulberry and plant mulberry proteins. At the same time, a certain part of the proteins (15%) is produced in the body of larvae while forming of cocoon , i.e., when the larvae of the mulberry silkworm no longer takes food. At the first age the mass of glands of larvae of a mulberry silkworm is 4% of its weight, at the fifth age this indicator already reaches 25-26%,

and the length of silk glands exceeds the length of the larvae five times.

To determine the number of proteins in the hemolymph on the third day of the fifth age, 10 larvae from each variant were weighed each day before the cocoons were formed, and silk glands were also separately weighed. The mass of larvae of mulberry silkworm and silk glands gradually grows from the third day of the fifth age to the eighth day.

As it turns out from the results of the experiment, the reason for increasing silkiness is the intense growth of silk glands, and the accumulation of a large amount of silk mass in them. The results of the experiment are shown in Table 3.

Table 3. *Amount of protein in hemolymph of silkworm, %.*

Sex of the larvae / Breeds	3 averages of the experiment			
	Sex of the larvae		Sex of the larvae	
	♀	♂	♀	♂
Digomi 1	8.5	8.2	8.7	8.3
Digomi 1_1	8.9	8.4	9.4	8.8
Digomi 2	10.6	8.7	11.3	9.3
Digomi 2_2	12.3	9.2	12.8	9.8

As can be seen from Table 3, the increased total protein content of Digomi 1_1 and Digomi 2_2 hemolymph gave better results than Digomi 1 and Digomi 2. This indicates that the selection of silkworm larvae by this method contributes to the synthesis of proteins in the body, which subsequently affects the increase in silkiness of silkworm cocoons. At the same time, the regularity of the content of proteins depending on sex remains, of course, the number of proteins in the hemolymph of female larvae of mulberry silkworm is much higher compared to the male ones.

Table 4. *Effect of the amount of total proteins in hemolymph (♀♂average) on some biological indicators of mulberry silkworm.*

Breeds	Number of total proteins, %	Silkiness of the cocoon, %	Number of grains in clutch, pcs
Digomi 1	8.3	23.6	670
Digomi 1_1	8.6	24.1	690
Digomi 2	9.6	24.8	712
Digomi 2_2	10.7	25.3	789

As the data of Table 4 show, there is a clear difference between the two experimental breeds of mulberry silkworm, moth fertility and the content of common proteins in hemolymph. For example, with an average protein content of 10.7%, respectively, the average silkiness of individuals of both breeds is higher, as well as the average number of eggs in the grain (790 pcs).

The purpose of the experiment was to establish a relationship between the concentration of common proteins in the hemolymph of larvae at the end of the fifth age, silkiness and fertility of the moth.

Based on the analysis of the data of table 4, a correlation was revealed between the concentration of common proteins in the hemolymph of silkworm larvae, the silkiness of the fresh cocoon, as well as the fertility of the moth. In particular, in Digomi 1 hemolymph, the content of total proteins in hemolymph is 12.3%, the average amount of fertilized eggs (laid by one moth) is 789 pcs, which is 77-119 pcs more compared to other versions.

Therefore, the greater the number of total proteins in the hemolymph, the higher the silkiness of the live cocoon of the mulberry silkworm and the fertility of the moth.

Conclusions

1. As a result of selection work, improved lines of mulberry silkworm Digomi 1_2 and Digomi 2_2 with high biotechnological properties, which are relatively resistant against various diseases of mulberry silkworm and are considered promising breeds, are obtained by the method of rapid improvement.
2. The silkworm breeds Digomi 1_2 and Digomi 2_2,

which originated from the breeds of Digmuri group, mainly contain medium size cocoons and are characterized by high biotechnological properties. Especially they have high indicators for the length of the cocoon thread, which is more than 2000 m.

3. The silkworm breeds Digomi 1 and Digomi 2, which originated from the breeds of Digmuri group and the evolved breeds Digomi 1_2 and Digomi 2_2 are valuable starting materials for producing even more highly productive breeds and production hybrids.
4. The mass of silky glands from the third day to the eighth day of the fifth age increased 4-7 times and amounted to 22.3-29.2% of the mass of mulberry silkworm, which accordingly affected the silkworm of a fresh cocoon, there is a direct connection between the mass of silkworm glands and the mass of silk in the cocoon, which can be used in the selection work of mulberry silkworm.

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Computer modeling and forecast of expected debris flow in the mletiskhevi

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A B S T R A C T

The aim of the research is computer modeling and risk analysis of debris flow of expected erosion and landslide genesis in Mletiskhevi, for which, at the initial stage, the minimum conditions for the resistance of the landslide slope mainly caused by debris flow were determined. In case of both dry and water-saturated ground.

At a later stage for modelling the debris flow we used computer program RAMMS (Rapid mass movement simulation) and for its functioning Mletiskhevi DEM (Digital Elevation Model) was used. Besides the inputs were defined for RAMMS.

As a result by modelling carried out with RAMMS computer program, the forecasting characteristics of the expected debris flow in the Mletiskhevi catchment area were defined: height, speed, pressure and volume of the extracted mass. Accordingly, debris flow risk zones were also identified. There are 3 houses, agricultural land, St. George Church and Motor bridge. Therefore, effective anti-debris flow measures should be taken immediately to minimize the risk of expected debris flow in Mletiskhevi.

Key words*:* Debris flow, erosion, landside, modelling, RAMMS.

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INTRODUCTION

From the natural disasters that occur in Georgia, special attention is paid to erosion and landslide genesis debris flow phenomena. There are 3000 debris flows in Georgia, which is about 29% of the country's territory. The population, strategic values like bridges, transport mains, water and energy objects, churches and other cultural monuments are at risk for being under the threat of debris flow [1].

The debris flows are created with special frequency in the waters of the MletisKhevi watershed (42˚02'09.70»N; 44˚45'38.87»E) basin tributary of river Aragvi (Fig. 1) in the Dusheti municipality (Georgia), where because of intensive exogenous catastrophic debris flow processes are creating. These processes threaten the Mleta village, tourist routes, Mleta St. George Church, motorway and bridge [2-4]. They also prevent the normal function of Tbilisi water supply - Zhinvali water reservoir. The debris flow mass formed in the Mletiskhevi ravine meets the river via the river Tetri Aragvi thus it flows into the Zhinvali water reservoir. The quality of the water reservoir is deteriorating and its useful

volume decreases within the short period of time, consequently restricting Tbilisi water supply.

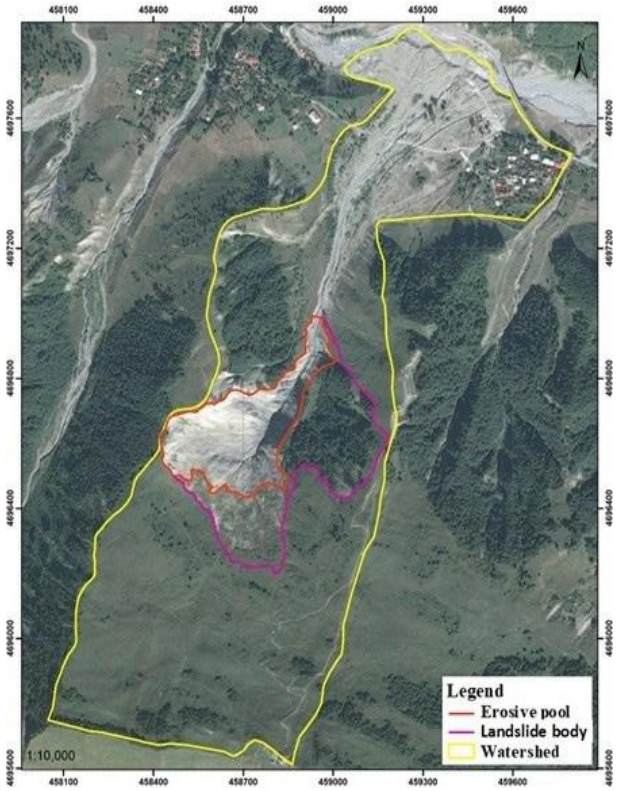

Fig. 1. *Mletiskhevi catchment area*

It should be noted that, in recent years, large-capacity debris flows (1953, 1981, 1982, 1983, 1985, 1987, 1989, 2001, 2003, 2005, 2011) have been observed in the Mleta gorge, as well as an increase in the frequency of debris flows, in particular, from 1897 to 2011, in Mleta, catastrophic debris flow were recorded 150 times, which resulted in the destruction of settlements and various objects. Field research has shown that not only has the frequency of debris flows increased, but also the volume of solid mass emitted by debris flows, the volume of disposable material often exceeding 1 million m^3 [5].

The situation has become especially vulnerable during the last five years, as the debris flow of mullet is becoming more frequent. A prominent example is the tragedy formed on Mletiskhevi on 10th August, 2018 which caused significant damage to 8 populated areas [6].

The average annual rainfall in Mletiskhevi is 1,339 mm. The maximum value of incoming precipitation is recorded in spring and summer: in May, June and July, when 1/3 of the total annual precipitation falls. The maximum average daily precipitation is within 45.6 mm, while the absolute maximum daily precipitation is 125.0 mm [7].

Mleta gorge is presented as a canyon with a slope of 500÷600 m and it decreases to 450÷200 m, the width of the bed bottom increases from 4÷5 m to 10÷15 m from the end of the transit zone. The output cone is presented in the form of an expanded cone up to 600÷800 m, its surface is relatively convex and is represented by sand-gravel and small fractions[8].

The geological structure of Mletiskhevi is composed of the lower valangin black shale (K_{1V1}), which is represented by limestone interlayers (0.5-1.2 m) with black shale marls (3-6 m). The azimuth of the layer direction is southeast 120-125°, the angle of inclination is 54-55°. The main rocks in the lower part of the slope are covered with strong (5-20 m) proluvial (pQ_{IV}) sediments and are represented by 20-25% gravel soil with clay filler, while the upper part is covered with eluvial-deluvial layers, whose power is variable:: reaches 4-8 m in the lower and upper parts of the slope, while 1.0-3.0 m in the main central part. They are represented by loamy soil with up to 30% crushed stone inclusions (edQ_{IV}) [5].

Objectives and Methods

The aim of the research is computer modeling and risk analysis of debris flow of expected erosion and landslide genesis in Mletiskhevi.

Landslide processes are the main provoking factor of debris flows in Mletiskhevi, it is the main supplier of solid material in the ravine, which determines the frequency of debris flows and its destructive force.

Landslide processes are the main provoking factor of debris flows in Mletiskhevi, it is the main supplier of solid material in the ravine, which determines the frequency of debris flows and its destructive force, accordingly, at the initial stage, field, laboratory and theoretical studies were conducted to assess the minimum conditions for the sustainability of the landslide slope in the Mletiskhevi gorge [9].

As a result of field studies in Mletiskhevi, it was found that the above-mentioned landslide is circus-shaped (Fig. 2), length 200-250m, width 250-300m, slope angle 250-500, landslide capacity 4-10 m, the surface is stepped, with open cracks, Alluvial-deluvial rocks float on the main rocks.

Fig. 2. *Landslide areas in Mletiskhevi*

To determine the landslide slope sustainability conditions, ground samples were taken from the landslide slope and the calculation parameters[10] were determined under laboratory conditions (Table 1).

Table 1. *Results of laboratory study of ground samples taken from the landslide slope of Mleta gorge*

Internal friction angle of the ground φ^0	Clutch c (t/m^2)	Porosity N %	Mineral density ρ	Water density ρ
23,0	25,0	0,42	2,72	1,0

The above data were used to determine the critical depth of the soil layer on the study slope (when movement begins) in the case of "dry" ground; The calculation was performed using the following methodology [3,4]:

$$\frac{1}{\bar{z}} \le \frac{c}{\rho g z} = \sin\alpha - \tan\varphi\cos\alpha, \quad (1)$$

where $\bar{z}=\frac{\rho g z}{c}$ -the relative thickness of the ground layer, the increase of which causes the slope to climb; ρ – density g acceleration of the force of gravity, z - thickness of the ground layer, φ- internal friction angle of the ground, α - the angle of inclination of the slope, and in the case of a slope saturated with water we will have:

$$\frac{1}{\bar{z}_1} \le \frac{c}{\rho g z_1} = \left(1-\frac{\rho_w}{\rho_m}\right)\cdot(\sin\alpha - \tan\varphi\cos\alpha) + \frac{\rho_w}{\rho_m}\cdot\sin\alpha\cdot\frac{1}{1-n} \quad (2)$$

In case of taking into account the results of laboratory research of ground samples taken from the landslide slope of Mleta gorge in the mentioned methodology, we get the following relations:

$$\frac{1}{z_1} = \left(1-\frac{1}{2{,}72}\right)(sin\alpha - 0{,}42cos\alpha) + \frac{1}{2{,}72}sin\alpha\frac{1}{1-0{,}43} = 0{,}63\sin\alpha - 0{,}26\,cos\alpha + 0{,}64\,sin\alpha$$

$$= 1{,}27\sin\alpha - 0{,}26\,cos\alpha \quad (3)$$

Finally we will have:

$$\frac{1}{\bar{z}} = \sin\alpha - 0.42\cos a \quad (4)$$

$$\frac{1}{\bar{z}_1} = 1.27\sin\alpha - 0.26\cos a \quad (5)$$

In the above relationships, the relationship between the critical slope relative depths and the slope is given in Fig. 3.

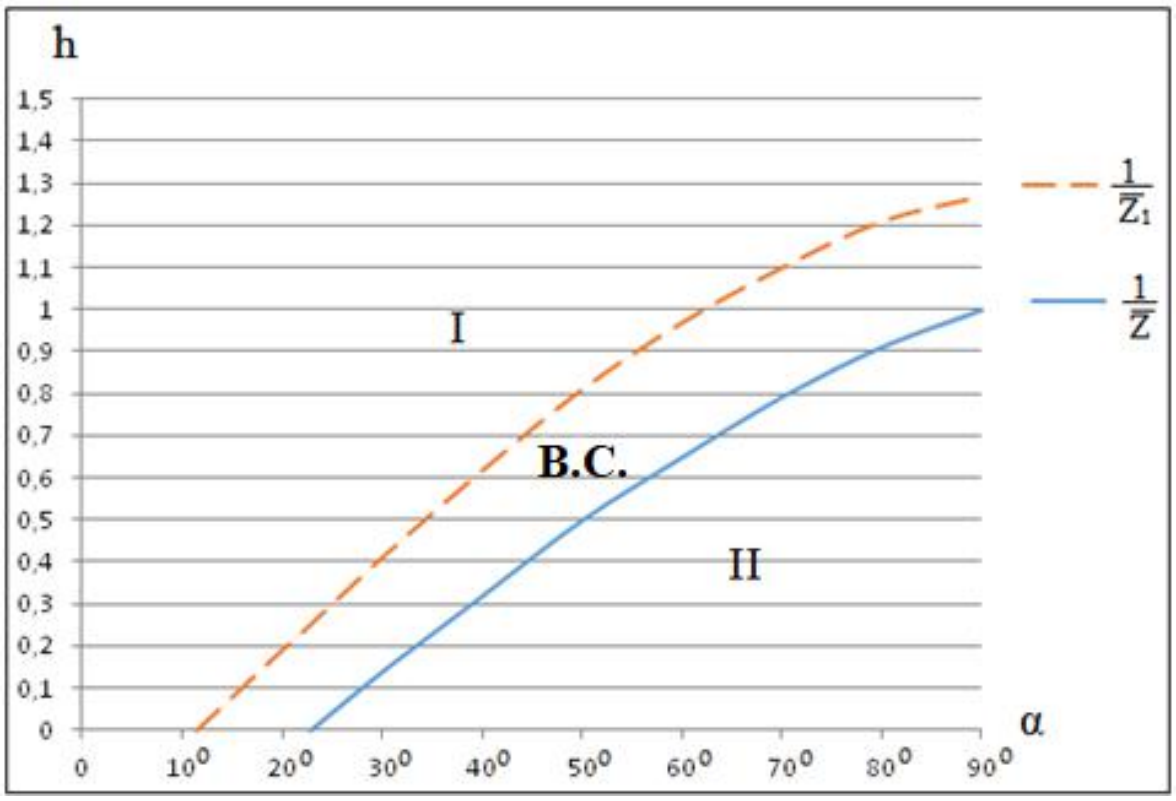

Fig. 3. *The critical ratio of the slope to the depths and slopes graph of the relationship between*

In Fig. 3, **(I)** indicate the steady state of the slope, (**B.C.**) Corresponds to the boundary condition, and **(II)** even beyond the boundary, i.e. when the slope starts to move.

Fig. 3 accordingly, water saturation reduces the critical angle of inclination of the slope (when movement begins) by about $12^0 \div 28^0$.

Get it for "dry" ground $\rho = 1.5t/m^3$, then $\alpha = 30^0$- in the case of $\frac{c}{\rho g z} \leq 0.13$, from where $z \geq 1{,}28$ m and start moving. In the case of $\alpha = 40^0$, $\frac{c}{\rho g z} \leq 0.32$, from where $z \geq 0{,}52$ m in the case of $\alpha = 50^0$, $\frac{c}{\rho g z} \leq 0.5$, from where $z \geq 0.33$ m.

For a water-saturated ground, motion will start at $\alpha = 30^0$ and $z_1 \geq 0{,}61$m, in the case of 40^0, $z_1 \geq 0{,}40$ m, while in the case of 50^0, $z_1 \geq 0{,}31$m.

The results of the report show that the landslide slope is unstable, the formation of large-capacity landslides is expected and provoke debris flow.

Based on the above, it is realistic for catastrophic debris flow to develop again in Mletiskhevi, which makes it necessary to determine the expected magnitudes and zones of influence of the expected debris flow, to assess the impact of debris flow on settlements and infrastructure, for which RAMMS was used [11].

Mletiskhevi DEM (Digital Elevation Model - 2x2 m resolution) was used for modeling and forecasting the expected debris flow in RAMMS (Fig. 4).

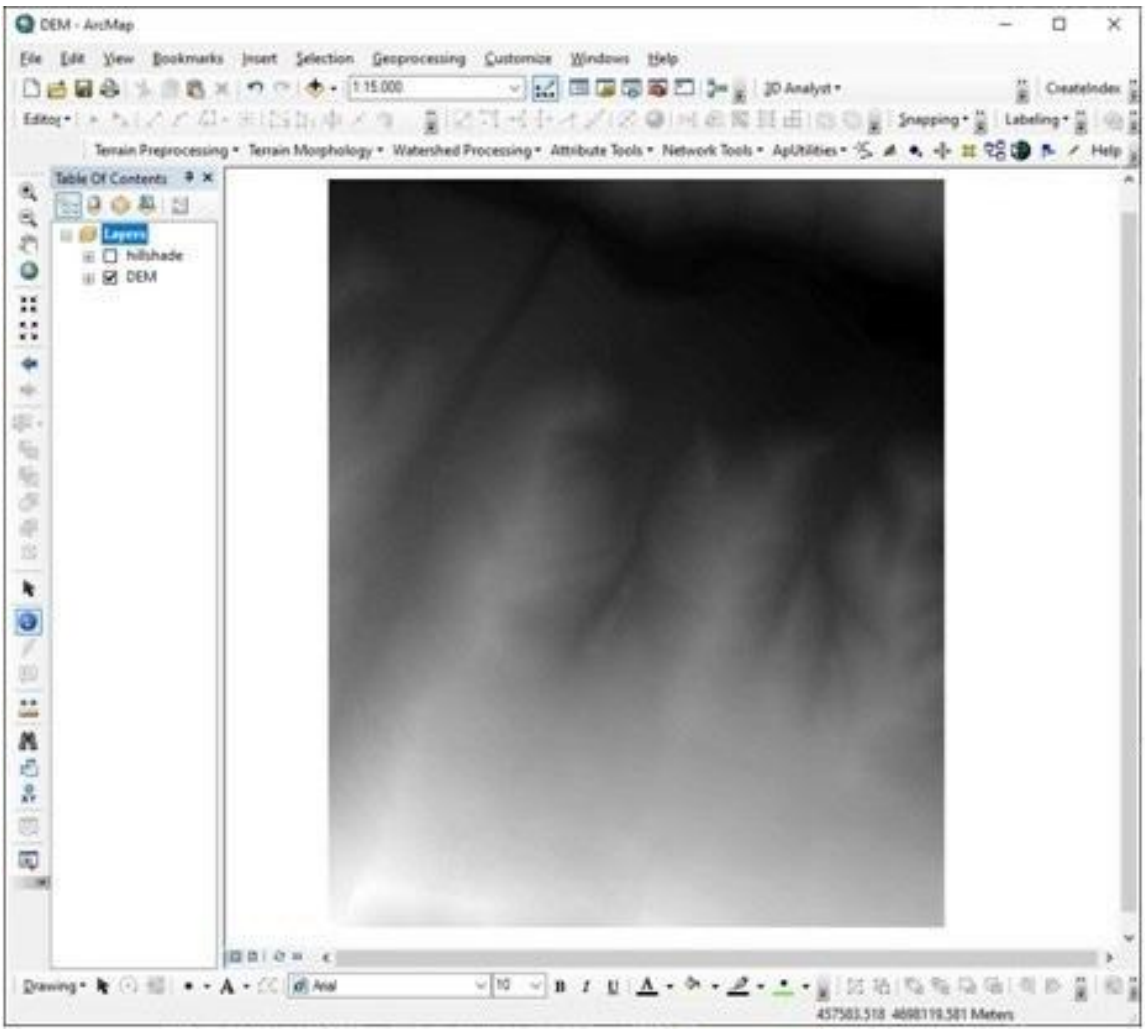

Fig. 4. *DEM (Digital Elevation Model) of Mletiskhevi*

Field surveys in Mletiskhevi revealed average depths and areas of the vulnerable district (landslide slope, eroded slope and bed accumulation zones) causing debris flow.

Due to the lack of previous measurements, the Voellmy friction model calibration took into account the maximum soil-ground volume (more than 1000000 m^3) produced by the debris flow formed in 1987 in Mletiskhevi.

To find the best- fit Voellmy friction coefficients (dry-Coulomb type friction μ and viscous-turbulent friction ξ) recommendations of the authors of ramms [11].

In view of the above, the processed initial data were entered into RAMMS and was modeled the expected catastrophic debris flow in Mletiskhevi (Table 2).

Table 2. *Basic settings to be entered in the computer program RAMMS*

The depth of soil-ground on the erosive slope (m)	The depth of soil-ground accumulated in the debris flow course (m)	The depth of landslide (m)	Debris flows density (kg/m^3)	Coefficient of Coulomb friction μ	Coefficient of turbulent viscosity ξ (m/sec^2)	Earth-pressure coefficient Λ	H cutoff (m)
0,2	2,0	5.8	2000	0,1	120	1	0.0001

Results and discussion

As a result of modeling performed with RAMMS, the maximum height (Fig. 5), velocity (Fig. 6), pressure (Fig. 7).

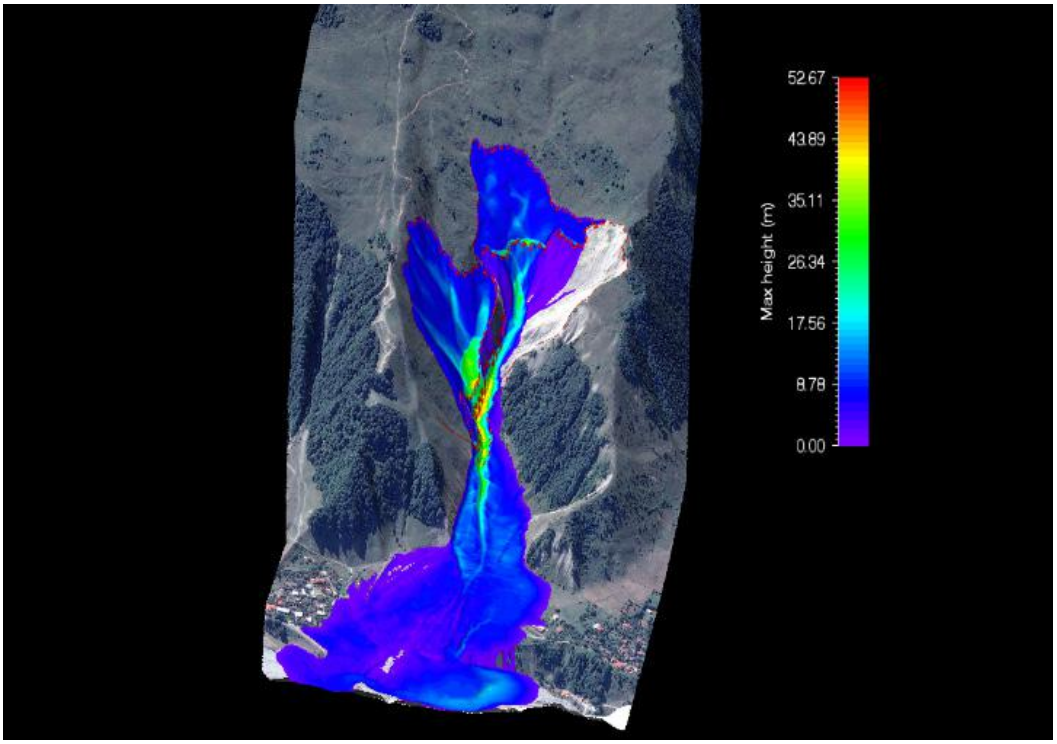

Fig. 5. *Maximum heights of expected debris flow in Mleta gorge*

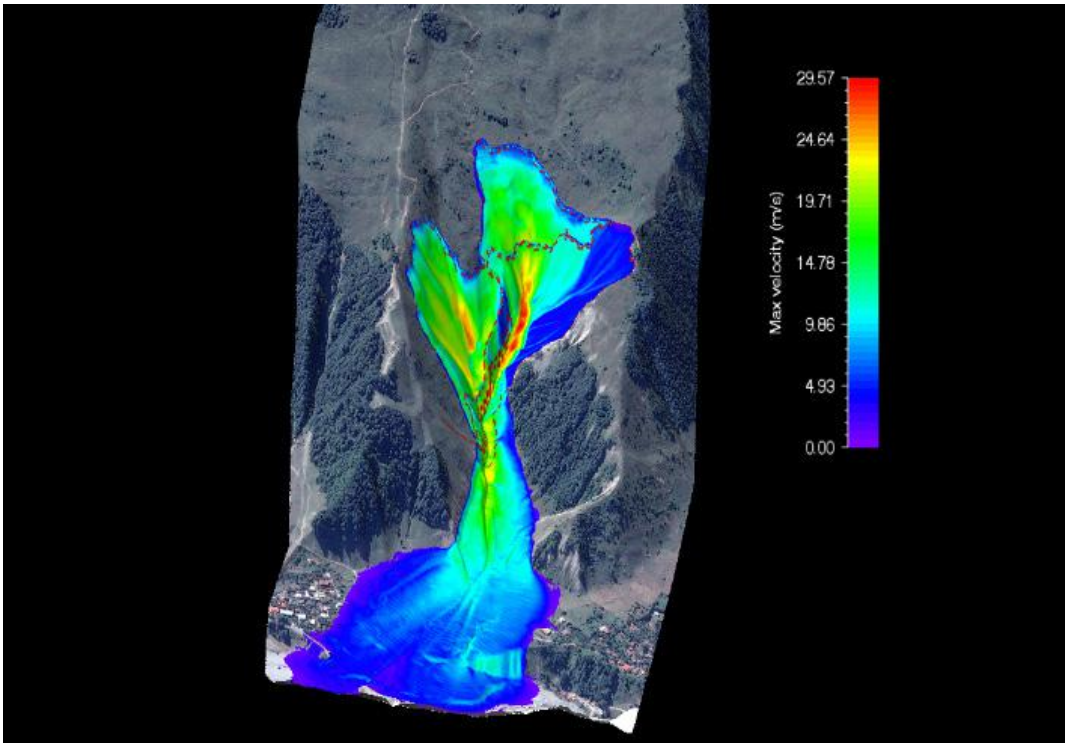

Fig. 6. *Maximum debris flow velocities in Mleta gorge*

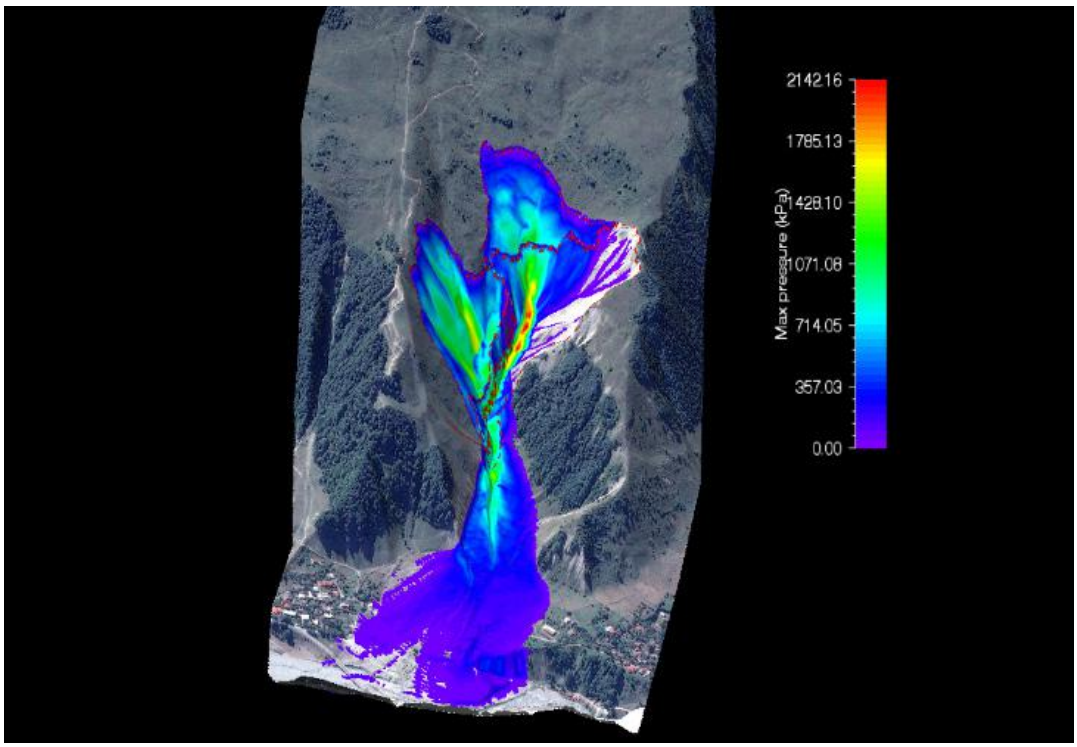

Fig. 7. *Maximum pressures of expected debris flow in Mleta gorge*

After the formation of the flood, the longitudinal profile of river Mletishkevi (Fig. 8) was also determined.

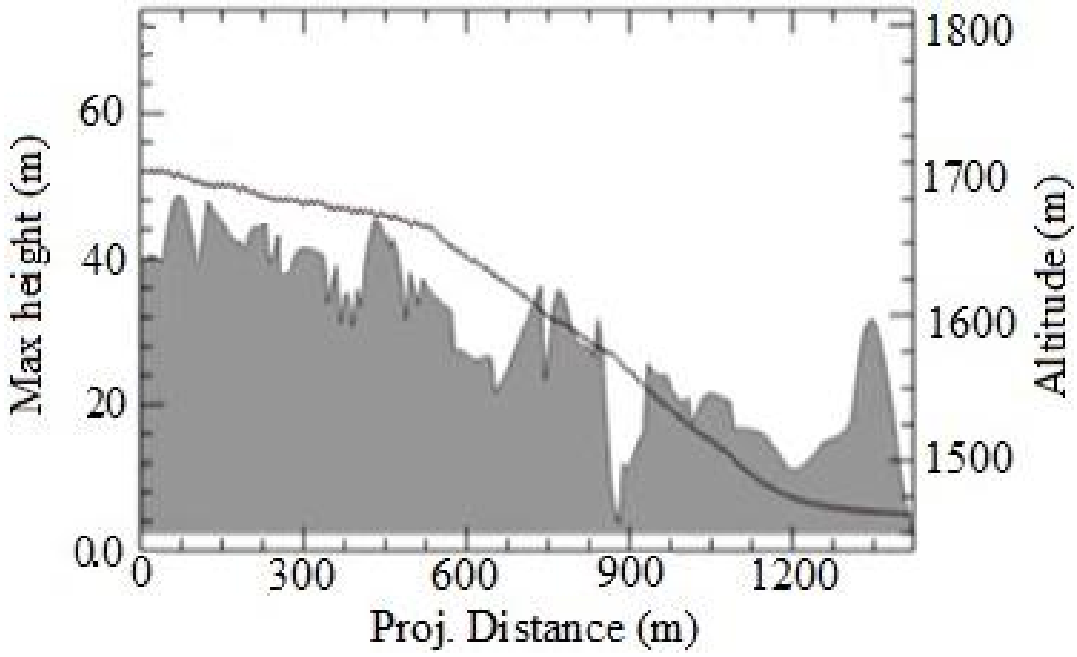

Fig. 8. Longitudinal profile of the river Mletiskhevi after forming the debris flow

Computer modeling also identified the expected debris flow zones in Mletiskhevi and the infrastructure of various destinations in the risk zone (Fig. 9).

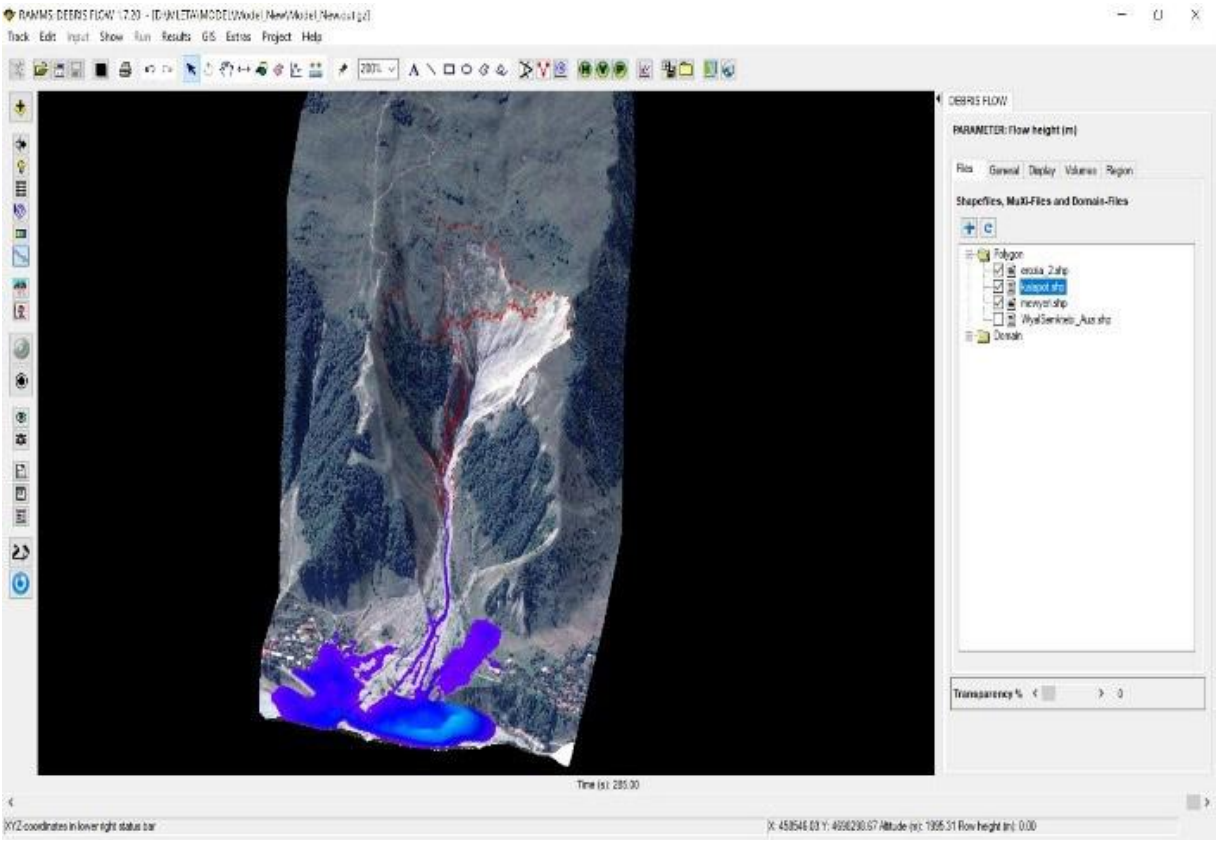

Fig. 9. *The final stage of modeling*

Numerical characteristics of the expected debris flow modeling with RAMMS in Mletiskhevi are given in Table 3.

Table 3. *Data obtained as a result of modeling performed by RAMMS*

The volume of the erosive mass mass on the slope m^3	The accumulated volume of the soil and ground in the debris flow source area m^3	The volume of landslide body m^3	Debris flows volume (m^3)	Debris flows MAX velocity (m/s)	Debris flows MAX flowheight (m)	Debris flows MAX pressure (kPa)
31888.9	6505.8	1063640	1102033.13	29.5694	52.6722	2142.16

Assessing the minimum conditions for the predominantly provocative landslide slope of the expected debris flow in Mletiskhevi, it was found that the study slope is dangerous for landslides, because in the case of an average slope of 30^0, a ground mass of 0.61 m is sufficient to withstand water saturation. Violation, and if we take into account that the depth of the landslide slope is up to 10 meters in most places, the absolute maximum daily precipitation during the peak period is equal to 125.0 mm and the slope angle varies from 200-500, then it can be said that at the head of Mleta gorge The risk of developing debris flow, the main provocative landslide processes, is high.

Also defined, during the modeling of the expected debris flow in Mletiskhevi, the maximum height of the expected debris flow front - 52.6722 m (narrow intersection of the river bed), the maximum speed of the debris flow - 29.5694 m/s, the pressure - 2142.16 kPa and the volume of the mass produced by the debris flow - 1102033.13 m^3, which is almost identical to the solid mass characteristics produced by the debris flow formed in Mletiskhevi in june 2-3, 1987.

Conclusions

The expected flood zones in Mletiskhevi, which includes some settlements, a church, a highway and a bridge, were also identified. The debris flow is likely to block the Tetri Aragvi riverbed and pose a flood risk to the population of Zemo Mleta. It is also expected to enter the Zinvali Reservoir from Mletiskhevi through the Tetri Aragvi riverbed, as the river Tetri Aragvi is the main supply artery for the Zinvali Reservoir. That will cause the limited water supply to Tbilisi, the capital of Georgia.

Based on the performed modeling data, it can be said that the expected ecological threat in Mletikhevi was predicted, which indicates the need for urgent implementation of optimal engineering environmental measures to regulate the expected ecological risks in Mletiskhevi.

Acknowledgment

This work was supported by the Shota Rustaveli National Science Foundation of Georgia (SRNSFG) [grant number AR 18-1491], for which the authors are grateful.

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Third contibution to the sawflies (Hymenoptera:Symphyta) of Kintrishi Nature Reserve (Georgia, Sakartvelo)(Part III)

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A B S T R A C T

2 482 specimen belonging to 89 sawflies species are listed from the 1 264 m altitude of Kintrishi National Park. Characteristic species are: *Onycholyda trigaria* (Konow, 1897), *Birka catellata* (Konow, 1900), *Strongylogaster caucasica* Schaposchnikov, 1885, *Eutomostethus ephippium* ssp. *vopiscus* (Konow, 1899), *Macrophya hamata* ssp. *caucasicola* Muche, 1969, *Sciapteryx byzantina* Benson, 1968, *Tenthredo albopicta* Puls, 1870, *Tenthredo purpurea* Puls, 1870, *Tenthredo radoszkowskii* (André, 1881), *Tenthredo shaposhnikovi* (Dovnar-Zapolskij, 1930), *Tenthredo talyshensis* Zhelochovtsev, 1988 and *Tenthredopsis viridis* Zhelochovtsev, 1941. Zoogeoɢraphic distributions of sawflies were analyzed and discussed. The known number of sawflies increased up to 142 in Kintrishi National Park. Twenty species are new records for the fauna of Georgia. Six genera *Periclista* Konow, 1886, *Amauronematus* Konow, 1890, *Eupontania* Zinovjev, 1985, *Euura* Newman, 1837, *Phyllocolpa* Benson, 1960 and *Pontania* Costa, 1852.

Key words: Sawflies, Sakartvelo, Georgia, Caucasus, Kintrishi National Park Hymenoptera, Symphyta, nature conservation, new records

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Introduction

Our present paper is the third part [1,2] of a series to investigate the sawfly (Hymenoptera: Symphyta) fauna of Kintrishi National Park. In the first paper [1], we studied the fauna of the lower altitudes, in the second part, the high altitudes [2] and in the present part that altitude of Kintrisi Nat. Res. where the most diverse sawfly fauna was detected.

Methods and material

The sawfly material was collected by using Malaise traps located at 1264 m altitude above sea level, at coordinates: N 41°44’38.8824; E42°5’0.2904 (approximately 6.03 km E of Didvake village). These altitudes (1, 264 above sea level) is the zone from the low subalpine Colchis mixed forest belt with dominance of beech (*Fagus orientalis*) and spruce (*Picea orientalis*). Other plants distributed in this altitude are *Castanea sativa*, *Carpinus caucasica*, *Tilia caucasica*, *Rhododendron ponticum*, *Rh. luteum*, *Rh. ungernii*, *Rh. caucasicum*, *Vaccinium arctostaphylos*, *Alnus barbata*, *Rumus* spp., *Polystichum* spp. and others (Figs 1 and 2). The average temperature is 8.5-9 °C. The coldest month is January - with an average temperature of 0.5 °C. The absolute minimum temperature ranges from -16-17 °C. The warmest month is August, with an average temperature of 15-15.5 °C. The annual amount of precipitation is 3 000 mm.

A Malais trap (Fig.3) was used to sample sawflies and was in operation from 20 April to 21 September 2018. The trap were checked in every 12-17 days and insects were removed. The material was preserved in alcohol, later, in 2022, the sawflies were mounted and genitalia were dissected for further identification. The fo llowing keys were used for species identification: Zhelochovtsev's work [3] on the sawflies of the European part of the former USSR, Lacourt´s manual [4] on the identification of European sawflies, Robert Benson´s monograph [5] on the Turkish sawfly fauna, Gussakovsky's monographs [6,7] on the Symphyta of the former USSR and the latest monograph on Czech and Slovakian sawflies [8]. We also used some recent revisions and works to make the identifications and biological data even more accurate [9,10,11,12,13]. Voucher specimens are deposited in the entomological collection of the Institute of Entomology of the Agricultural University of Georgia. For the discussion of the distribution of sawflies, we have consulted Roller and Haris entitled Sawflies of the Carpathian Basin, History and Current Research [14] the most recent European checklist of species [15] and the monograph by Sundukov [16] on the sawflies of Russia. The nomenclature used in this article, follows the latest monograph of the European sawflies [4] with special attention to the subfamily Nematinae and corrects the conclusions of Prous et al. [17]. New country records are indicated with an asterisk. The higher classification of sawflies used in this paper follows the Hymenoptera section of Fauna Europaea [18]. For host plant records books and papers of Macek et al. [8] and Martynov et al. [19] were consulted.

List of species

Family - Argidae

Genus *Arge* Schrank, 1802

1. *Arge berberidis* Schrank, 1802: 01-15. 06. 2018, 1 male. Frequent. Larva on *Berberis* and *Mahonia* spp. Palaearctic.

2. *A. cyanocrocea* (Forster, 1771): 20. 04. - 05. 05. 2016, 7 males, 05-20. 05. 2018, 1 female, 19 males, 01-15. 06. 2018, 7 males, 15-29. 06. 2018, 3 males, 29. 06. - 13. 07. 2018, 2 males, 13-27. 07. 2018, 1 female, 13 males. (This colour variation, formerly classified as Arge syriaca (Mocsáry, 1880)). Common, West Palaearctic species. Known host plants: *Rubus idaeus* and *Sanguisorba officinalis*.

3. *A. fuscipes* (Fallén, 1808): 20. 05. - 01. 06. 2018, 1 female, 01-15. 06. 2018, 1 female. Sporadic, Palaearctic species. Host plants: *Betula* spp. and *Salix caprea*.

Genus *Sterictiphora*

4. *Sterictiphora longicornis* Chevin, 1982: 20. 04. - 05. 05. 2016, 1 female. Sporadic. Host plant: *Carpinus betulus*. Adults associated with *Prunus spinosa*. West Palaearctic species

Family - Cephidae

Genus *Cephus* Latreille, 1803

5. *Cephus spinipes* (Panzer, 1800): 05-20. 05. 2018, 2 females. Frequent. Host plant: *Phleum pratense*. Palaearctic.

Genus *Janus* Stephens, 1829

6. *Janus luteipes* (Lepeletier, 1823): 20. 05. - 01. 06. 2018, 1 female. Host plants: *Salix*,

Populus and *Viburnum* spp. Sporadic, Palaearctic species.

Family - Orussidae

Genus *Orussus* Latreille, 1797

7. *Orussus abietinus* (Scopoli, 1763): 20. 04. - 05. 05. 2016, 1 male. Sporadic, Palaearctic species. Parasitoid of *Semanotus unduatus* L.

Family - Pamphiliidae

Genus *Onycholyda* Takeuchi, 1938

8. *Onycholyda trigaria* (Konow, 1897): 20. 04. - 05. 05. 2016, 1 male, 05-20. 05. 2018, 1 female, 7 males, 20. 05. - 01. 06. 2018, 1 female, 2 males. Frequent, Ponto-Caspian-Iranian species. Hostplant unknown.

Genus *Pamphilius* Latreille, 1803

9. *Pamphilius ignymontiensis* Lacourt, 1973: 05-20. 05. 2018, 2 males. Sporadic. Known host plants: *Acer platanoides* and *A. campestre*. West Palaearctic species.

10. *Pamphilius sylvaticus* (Linné, 1758): 20. 04. - 05. 05. 2016, 1 male, 20. 05. - 01. 06. 2018, 2 females. One of the most frequent sawfly species. Host plants: *Sorbus aucupariae*, *Malus* spp., *Prunus* spp. and *Crataegus* spp. West Palaearctic species.

Family - Tenthredinidae

Subfamily - Dolerinae

Genus *Dolerus* Jurine, 1807

11. *Dolerus* (*Dicrodolerus*) *vestigialis* (Klug, 1818): 05-20. 05. 2018, 1 female, 20. 05. - 01. 06. 2018, 1 female, 01-15. 06. 2018, 1 female. Common, Palaearctic species. Host plants: *Equisetum palustre*, *E. sylvaticum*, *E. arvense* and *E. pratense*.

Subfamily - Selandrinae

Genus *Aneugmenus* Hartig, 1837

12. *Aneugmenus coronatus* (Klug, 1818): 20. 04. - 05. 05. 2016, 1 female, 05-20. 05. 2018, 10 females, 20. 05. - 01. 06. 2018, 5 females, 01-15. 06. 2018, 9 females, 1 male, 15-29. 06. 2018, 8 females, 29. 06. - 13. 07. 2018, 4 males, 13-27. 07. 2018, 4 females, 27. 07. - 20. 08. 2018, 1 female, 20. 08. - 07. 09. 2018, 1 female. Sporadic, Palaearctic species. Larva on *Dryopteris filix-mas*, *Aspidium* sp., *Athyrium filix-femina* and *Pteridium aquilinum*.

13. *Aneugmenus padi* (Linné, 1760): 01-15. 06. 2018, 6 females, 69. 15-29. 06. 2018, 1 female. Host plants: *Asplenium* sp. and *Pteridium aquilinum*. Sporadic. West Palaearctic species; introduced to North America.

Genus *Birka* Malaise, 1944

14. *Birka* (*Birka*) *catellata* (Konow, 1900): 20. 04. - 05. 05. 2016, 1 female, 42 males, 05-20. 05. 2018, 2 females, 47 males, 20. 05. - 01. 06. 2018, 4 males, 01-15. 06. 2018, 14 males, 15-29. 06. 2018, 17 males, 13-27. 07. 2018, 3 females, 23 males, 29. 06. - 13. 07. 2018, 6 females, 20 males, 27. 07. - 20. 08. 2018, 1 female, 4 males, 20. 08. - 07. 09. 2018, 1 female. Ponto-Caspian and Turanian. Common species. Hostplant unknown.

15. *B.* (*Birka*) *cinereipes* (Klug, 1816): 20. 04. - 05. 05. 2016, 2 males. Frequent. Host plants: *Brachytecium reflexum*, *Ceratodon purpureus*, *Chenopodium album*, *Dicranum scoparium*, *Fragaria vesca*, *Hedwigia ciliata*, *Myosotis arvensis*, *Plagiomnium cuspidatum*, *Plagiothecium denticulatum*, *Polygonum aviculare*, *Polytrichum commune*, *Pseudobryum cinclidiodes*, *Sanionia uncinata*, *Stellaria media*, *Veronica chamaedrys* and *V. officinalis*. Holarctic.

Genus *Nesoselandria* Rohwer, 1910

16. *Nesoselandria morio* (Fabricius, 1781): 05-20. 05. 2018, 1 female. Frequent. Host plants: *Brachytecium reflexum*, *Ceratodon purpureus*, *Chenopodium album*, *Dicranum scoparium*, *Fragaria vesca*, *Hedwigia ciliata*, *Myosotis arvensis*, *Plagiomnium cuspidatum*, *Plagiothecium denticulatum*, *Polygonum aviculare*, *Polytrichum commune*, *Pseudobryum cinclidiodes*, *Sanionia uncinata*, *Stellaria media*, *Veronica chamaedrys* and *V. officinalis*. Holarctic.

Genus *Strongylogaster* Dahlbom, 1835

17. *Strongylogaster caucasica* Schaposchnikov, 1885: 20. 04. - 05. 05. 2016, 13 females, 50 males, 05-20. 05. 2018, 3 females, 1 male, 15-29. 06. 2018, 1 female, 1 male, 29. 06. - 13. 07. 2018, 1 female. Sporadic. Hostplants unknown. Ponto-Caspian.

18. *S. multifasciata* (Geoffroy, 1785): 20. 04. - 05. 05. 2016, 8 females, 05-20. 05. 2018, 11 females, 20. 05. - 01. 06. 2018, 1 female, 13-27. 07. 2018, 7 females. Frequent. Hostplants: *Dryopteris* sp., *Matteuccia struthiopteris*, *Aspidium* sp., *Polystichum* sp. and *Pteridium aquilinum*. Palaearctic.

Subfamily – Allantinae

Genus *Allantus* Panzer, 1801

19. *Allantus* (*Emphytus*) *cinctus* (Linné, 1758): 20. 04. - 05. 05. 2016, 57 males, 05-20. 05. 2018, 23 females, 115 males, 20. 05. - 01. 06. 2018, 11 females, 82 males, 04-15. 06. 2015, 1 254 m, 5 females, 70 males, 15-29. 06. 2018, 5 females, 83 males, 29. 06. - 13. 07. 2018, 7 females, 71 males, 13-27. 07. 2018, 4 females, 18 males, 27. 07. - 20. 08. 2018, 3 females, 9 males, 20. 08. - 07. 09. 2018, 4 females, 29 males, 07-21. 09. 2018, 4 females, 5 males. Common. Host plants: *Rosa* and *Fragaria* spp. Holarctic.

20. *A. (Allantus) togatus* (Panzer, 1801): 01-15. 06. 2018, 1 male. Sporadic, Palaearctic species. Larva on *Betula*, *Quercus* and *Salix* spp.

Genus *Ametastegia* Costa, 1882

21. *Ametastegia* (*Protemphytus*) *carpini* (Hartig, 1837): 20. 04. - 05. 05. 2016, 1 male, 05-20. 05. 2018, 1 male, 01-15. 06. 2018, 1 female. Frequent. Holarctic. Host plants: *Geranium* spp.

22. *A.* (*Ametastegia*) *equiseti* (Fallén, 1808): 20. 04. - 05. 05. 2016, 1 female, 20. 05. - 01. 06. 2018, 1 male. Frequent. Larva on *Chenopodium album*, *Lythrum salicaria*, *Polygonum persicaria* and *Rumex acetosella*. Holarctic.

23. *A.* (*Protemphytus*) *pallipes* (Spinola, 1808): 20. 05. - 01. 06. 2018, 1 female. Frequent. Host plants: *Viola* spp. Holarctic.

Genus *Athalia* Leach, 1817

24. *Athalia bicolor* Serville, 1823: 01-15. 06. 2018, 1 female. Frequent. Host plant: *Ranunculus* spp. Palaearctic.

25. *A. circularis* ssp. *circularis* (Klug, 1815): 20. 04. - 05. 05. 2016, 5 males,

05-20. 05. 2018, 1 female, 14 males, 01-15. 06. 2018, 1 female, 28 males, 15-29. 06. 2018, 2 females, 16 males, 13-27. 07. 2018, 8 females, 25 males, 29. 06. - 13. 07. 2018, 2 females, 9 males, 27. 07. - 20. 08. 2018, 2 males, 20. 08. - 07. 09. 2018, 1 female, 5 males. (This colour variation was formerly classified as *A. cordatoides* Priesner, 1928) Frequent. Host plants: *Arctium lappa*, *Ajuga reptans*, *Veronica beccabunga*, V. longifolia, *V. officinalis*, *Alliaria petiolata*, *Glechoma hederacea*, *Melampyrum*, *Capsella* and *Lycopus* spp. Palaearctic.

26. *A. cordata* Serville, 1823: 20. 04. - 05. 05. 2016, 1 female, 7 males, 05-20. 05. 2018, 1 male, 01-15. 06. 2018, 1 female, 29. 06. - 13. 07. 2018, 1 male. Common. Larva on *Misopates orontinum*, *Antirrhinum majus*, *Ajuga reptans*, *Teucrium scorodonia* and *Plantago* spp. West Palaearctic.

27. *A. cornubiae* Benson, 1931: 15-29. 06. 2018, 1 female, 70. 13-27. 07. 2018, 1 female, 29. 06. - 13. 07. 2018, 2 females, 20. 08. - 07. 09. 2018,1 female, 07-21. 09. 2018, 1 female. Local colour variation: clypeus and labrum light coloured, in European specimens, they are dark, brown. Shape of hypopygium is typical for *A. cornubiae* Bens. Sporadic, West Palaearctic species. Larva on *Sedum album*.

28. *A. liberta* (Klug, 1815): 20. 04. - 05. 05. 2016, 2 males, 05-20. 05. 2018, 3 females, 6 males, 01-15. 06. 2018, 20 males, 15-29. 06. 2018, 3 females, 14 males, 13-27. 07. 2018, 2 females, 4 males, 29. 06. - 13. 07. 2018, 6 males, 20. 08. - 07. 09. 2018, 1 male. Frequent, West Palaearctic species. Feeding on *Alliaria petiolata*, *Arabidopsis thaliana*, *Cardamine hirsuta* and *Sisymbrium officinale*.

29. *A. lugens* (Klug, 1815): 20. 04. - 05. 05. 2016, 1 male, 05-20. 05. 2018, 1 female, 15 males, 01-15. 06. 2018, 6 females, 87 males, 15-29. 06. 2018, 6 fe8 males, 61 males, 29. 06. - 13. 07. 2018, 3 females, 7 males, 13-27. 07. 2018, 4 females, 15 males, 27. 07. - 20. 08. 2018, 1 male, 20. 08. - 07. 09. 2018, 2 males, 07-21. 09. 2018, 1 male. Frequent, Palaearctic species. Hostplants: *Raphanus* spp., *Lepidium sativum*, *Cardamine* spp., *Brassica* spp., *Cruciferae*.

Genus *Empria* Lepeletier & Serville, 1828

30. *Empria longicornis* (Thomson, 1871): 20. 04. - 05. 05. 2016, 113 males, 05-20. 05. 2018, 15 females, 82 males, 20. 05. - 01. 06. 2018, 6 females, 9 males, 01-15. 06. 2018, 2 females, 15-29. 06. 2018, 1 female. Frequent, West Palaearctic species. Larva on *Rubus idaeus*.

31. *E. sexpunctata* (Serville, 1823): 20. 04. - 05. 05. 2016, 1 female, 8 males. Frequent, West Palaearctic species. Larva on *Geum* spp.

32. *E. tridens* (Konow, 1896): 20. 04. - 05. 05. 2016, 1 female, 20. 05. - 01. 06. 2018, 1 female. Frequent, Palaearctic species. Host plants: *Geum* spp. and *Rubus idaeus*.

Genus *Eriocampa* Hartig, 1837

33. *Eriocampa umbratica* (Klug, 1816): 20. 04. - 05. 05. 2016, 1 female, 4 males. Frequent. Larva on *Alnus glutinosa* and *A. incana*. West Palaearctic.

Genus *Monsoma* MacGillivray, 1908

34. *Monsoma pulveratum* (Retzius, 1783): 20. 04. - 05. 05. 2016, 1 female, 05-20. 05. 2018, 1 female. Frequent. Holarctic. Hostplants: *Alnus glutinosa* and *Alnus incana*.

Subfamily - Heterarthrinae

Genus *Caliroa* Costa, 1859

35. *Caliroa annulipes* (Klug, 1816): 15-29. 06. 2018, 1 male. Sporadic, Palaearctic species; introduced to Canada. Larva on Betula, Salix, Quercus, Tilia, Rosa spp. and Vaccinium myrtillus.

36. *C. cerasi* (Linné, 1758): 27. 07. - 20. 08. 2018, 1 male. Frequent. Larva on *Pyrus*, *Malus*, *Prunus*, *Crataegus*, *Sorbus*, *Rosa*, *Cydonia*, *Mespilus*, *Rubus*, *Amygdalus*, *Cerasus*, *Amelanchier*, *Pyracantha*, *Cotoneaster* rarely *Quercus* and *Salix* spp. Cosmopolita.

37. *C. cinxia* (Klug, 1816): 05-20. 05. 2018, 1 male, 01-15. 06. 2018, 1 male, 15-29. 06. 2018, 7 males. Sporadic, West Palaearctic species. Larva on *Quercus* spp.

38. *C. varipes* (Klug, 1816): 05-20. 05. 2018, 9 males, 20. 05. - 01. 06. 2018, 1 264 m 1 male, 01-15. 06. 2018, 3 males. Sporadic. Palaearctic. Larva on *Quercus*.

Genus *Endelomyia* Ashmead, 1898

39. *Endelomyia aethiops* (Gmelin, 1790): 20. 04. - 05. 05. 2016, 1 female. Sporadic. Larva on *Rosa* spp. Holarctic.

Genus *Heterarthrus* Stephens, 1835

40. *Heterarthrus vagans* (Fallén, 1808): 15-29.

06. 2018, 1 female. Sporadic, Palaearctic species. Host plants: *Alnus* spp.

Genus *Metallus* Forbes, 1885

41. *Metallus pumilus* (Klug, 1816): 05-20. 05. 2018, 3 males, 68. 01-15. 06. 2018, 22 males, 69. 15-29. 06. 2018, 3 males, 29. 06. - 13. 07. 2018, 2 males, 13-27. 07. 2018, 9 males, 29. 06. - 13. 07. 2018, 3 males, 27. 07. - 20. 08. 2018, 3 males, 20. 08. - 07. 09. 2018, 2 males. Common, Palaearctic species. Larva inside the leaves of *Rubus caesius* and *Rubus idaeus*.

Genus *Parna* Benson, 1936

42. *Parna tenella* (Klug, 1816): 20. 04. - 05. 05. 2016, 1 male, 05-20. 05. 2018, 2 females. Spo2 radic,West Palaearctic species. Larva inside the leaves of *Tilia* spp.

Subfamily - Blennocampinae

Genus *Claremontia* Rohwer, 1909

43. *Claremontia alternipes* (Klug, 1816): 20. 04. - 05. 05. 2016, 11 females, 42 males, 05-20. 05. 2018, 3 females, 7 males. Sporadic. Hostplant: *Rubus idaeus*. West Palaearctic.

Genus *Eurhadinoceraea* Enslin, 1920

44. *Eurhadinoceraea fulviventris* (Scopoli, 1763): 20. 04. - 05. 05. 2016, 76 males, 4 females, 05-20. 05. 2018, 9 females, 39 males, 20. 05. - 01. 06. 2018, 1 males, 01-15. 06. 2018, 8 males. Common. Hostplant unknown. Southern part of the Palaearctic region.

Genus *Eutomostethus* Enslin, 1914

45. *Eutomostethus ephippium* (Konow, 1899): 20. 04. - 05. 05. 2016, 13 males,

05-20. 05. 2018, 7 females, 4 males, 68. 01-15. 06. 2018, 1 female, 3 males, 13-27. 07. 2018, 1 female. Common. Hostplants: Poaceae. Ponto-Caspian subspecies.

Genus *Periclista* Konow, 1886

46. *Periclista* (*Periclista*) *lenta* Konow, 1903: 20. 05. - 01. 06. 2018, 1 male. Sporadic, West Palaearctic species. Larva on *Quercus* sp.

Subfamily – Nematinae

Genus *Amauronematus* Konow, 1890

47. *Amauronematus humeralis* (Serville, 1823): 20. 04. - 05. 05. 2016, 1 male. Sporadic, Palaearctic species. Larva on various Salix species like *Salix caprea*, *S. cinerea*, *S. aurita*, *S. fragilis* and *S. alba*.

Genus *Cladius* Illiger, 1807

48. *Cladius pectinicornis* (Geoffroy, 1785): 01-15. 06. 2018, 1 female. Common. Host plants: *Alchemilla*, *Filipendula*, *Fragaria*, *Potentilla*, *Sanguisorba*, *Rosa* and *Rubus* spp. Holarctic.

Genus *Eupontania* Zinovjev, 1985

49. *Eupontania pedunculi* (Hartig, 1837): 20. 04. - 05. 05. 2016, 1 male, 05-20. 05. 2018, 1 female, 01-15. 06. 2018, 1 male. Sporadic, Palaearctic species. Larva on *Salix aurita*, *S. caprea*, *S. starkeana* and *S. silesiaca*.

Genus *Euura* Newman, 1837

50. *Euura mucronata* (Hartig, 1837): 20. 04. - 05. 05. 2016, 1 male. Sporadic, West Palaearctic species. Host plants: *Salix aurita*, *S. caprea* and *S. cinerea*.

Genus *Hoplocampa* Hartig, 1837

51. *Hoplocampa plagiata* (Klug, 1816): 20. 04. - 05. 05. 2016, 13 females. West Palaearctic species. Sporadic. Larva on *Amelanchier ovalis*.

Genus *Nematinus* Rohwer, 1911

52. *Nematinus acuminatus* (Thomson, 1871): 15-29. 06. 2018, 1 female, 1 male. Sporadic, Palaearctic species. Larva on *Betula* spp.

Genus *Nematus* Panzer, 1801

53. *Nematus lucidus* (Panzer, 1801): 20. 05. - 01. 06. 2018, 7 males, 01-15. 06. 2018, 1 male, 01-15. 06. 2018, 5 males, 15-29. 06. 2018, 2 males. Frequent. Larva on *Crataegus* and *Prunus spinosa*. Palaearctic.

Genus *Pachynematus* Konow, 1890

54. *Pachynematus clitellatus* (Serville, 1823): 05-20. 05. 2018, 1 male. Frequent, Holarctic species. Larva on various Graminae.

55. *P. fallax* (Serville, 1823): 05-20. 05. 2018, 1 male. Frequent, West Palaearctic species. Host plants: various Poaceae and *Carex* spp.

Genus *Phyllocolpa* Benson, 1960

56. *Phyllocolpa leucapsis* (Tischbein, 1846): 05-20. 05. 2018, 1 female. Frequent, Holarctic species. Larva on *Salix cinerea*, *S. aurita* and *S. silesiaca*.

Genus *Pontania* Costa, 1852

57. *Pontania* (*Tubpontania*) *nudipectus* Vikberg, 1965: 05-20. 05. 2018, 1 female. Sporadic, West Palaearctic species. The gall on *Salix phylicifolia*.

Genus *Priophorus* Latreille, 1810

58. *Priophorus brullei* Dahlbom, 1835: 05-20. 05. 2018, 16 males, 20. 05. - 01. 06. 2018, 10 males, 01-15. 06. 2018, 1 female, 4 males, 15-29. 06. 2018, 1 264, 2 females, 4 males, 13-27. 07. 2018, 12 males, 29. 06. - 13. 07. 2018, 5 females, 12 males, 27. 07. - 20. 08. 2018, 6 males, 20. 08. - 07. 09. 2018, 2 males, 07-21. 09. 2018, 1 female. Frequent. Larva on *Rubus* spp. Cosmopolitan.

59. *P. compressicornis* (Fabricius, 1804): 20. 04. - 05. 05. 2016, 1 female, 3 males, 05-20. 05. 2018, 1 female, 13-27. 07. 2018, 1 female, 27. 07. - 20. 08. 2018, 1 female, 07-21. 09. 2018, 1 female. Frequent pest. Hostplants: *Betula*, *Cotoneaster*, *Prunus*, *Rubus*, *Sorbus*, *Fragaria*, *Crataegus*, *Corylus* and *Rosa* spp. Holarctic.

60. *P. rufipes* (Serville, 1823): 20. 04. - 05. 05. 2016, 1 female, 13-27. 07. 2018, 1 male. Sporadic, West Palaearctic species. Larva on *Ulmus* spp.

Genus *Pristiphora* Latreille, 1810

61. *Pristiphora* (*Pristiphora*) *aphantoneura* (Förster, 1854): 01-15. 06. 2018, 1 female. Sporadic, Palaearctic species. Host plant: *Lathyrus pratensis*.

62. *P.* (*Pristiphora*) *armata* (Thomson, 1863): 05-20. 05. 2018, 1 male, 13-27. 07. 2018, 1 male, 29. 06. - 13. 07. 2018, 1 male. Frequent, Palaearctic species. Host plants: *Crataegus* spp. and *Prunus avium*.

63. *P.* (*Pristiphora*) *leucopus* (Hellén, 1948): 20. 04. - 05. 05. 2016, 1 male, 05-20. 05. 2018, 1 male, 15-29. 06. 2018, 1 male, 20. 05. - 01. 06. 2018, 1 male, 01-15. 06. 2018, 1 female, 1 male, 27. 07. - 20. 08. 2018, 1 male. Frequent, West-Palaearctic species. Larva on Tilia spp.

64. *P.* (*Pristiphora*) *melanocarpa* (Hartig, 1840): 3-27. 07. 2018, 1 female. Sporadic, Holarctic sawfly. Host plant: *Betula* spp.

65. *P.* (*Pristiphora*) *pallidiventris* (Fallén, 1808): 20. 04. - 05. 05. 2016, 2 males, 20. 04. - 05. 05. 2016, 1 male. Frequent. Larva on *Geum*, *Potentilla*, *Rubus* and *Filipendula* spp. Holarctic.

66. *P.* (*Lygaeonematus*) *paralella* (Hartig, 1840: 05-20. 05. 2018, 1 female. Sporadic, West Palaearctic species.Larva on Picea abies.

Genus *Pteronidea* Rohwer, 1911

67. *Pteronidea* (*Pteronidea*) *dispar* (Zaddach, 1876): 13-27. 07. 2018, 1 male, 27. 07. - 20. 08. 2018, 1 male. Sporadic, Palaearctic species. Larva on *Betula* spp.

68. *P.* (*Pteronidea*) *fuscomaculata* (Förster, 1854): 13-27. 07. 2018, 1 male. Sporadic, West Palaearctic species. Host plants: *Populus tremula* and *Salix* spp.

69. *P.* (*Pteronidea*) *hypoxantha* (Förster, 1854): 15-29. 06. 2018, 1 male. Sporadic, Palaearctic species. Larva on *Salix fragilis*, *S. viminalis*, *S. aurita*, *S. cinerea*, *S. caprea*, *S. purpurea* and *Populus* spp.

70. *P.* (*Pteronidea*) *poecilonota* (Zaddach, 1876): 20. 04. - 05. 05. 2016 1 female. Sporadic, Holarctic species. Larva on *Betula* spp.

71. *P.* (*Pteronidea*) *sylvestris* (Cameron, 1884): 01-15. 06. 2018, 1 male, 15-29. 06. 2018, 1 male. Sporadic, Palaearctic species. Larva on *Salix caprea*, *S. pentandra* and *S. phylicifolia*.

Genus *Sharliphora* Wong, 1969

72. *Sharliphora nigella* (Förster, 1854): 20. 04. - 05. 05. 2016, 30 females, 27 males. Frequent, West Palaearctic species. Larva on *Picea* spp.

Subfamily - Tenthredininae

Genus *Macrophya* Dahlbom, 1835

73. *Macrophya* (*Macrophya*) *alboannulata* Costa, 1859: 20. 04. - 05. 05. 2016, 18 males, 05-20. 05. 2018, 1 female, 1 male, 20. 05. - 01. 06. 2018, 11 females, 16 males, 01-15. 06. 2018, 10 females, 7 males, 15-29. 06. 2018, 7 females, 6 males, 29. 06. - 13. 07. 2018, 1 female. Frequent, West Palaearctic species. Larva on *Sambucus nigra*, *S. racemosa* and *S. ebulus*.

74. *Macrophya* (*Macrophya*) *caucasicola* Muche, 1969: 20. 04. - 05. 05. 2018, 59 males, 05-20. 05. 2018, 6 females, 24 males, 01-15. 06. 2018, 16 females, 37 males, 15-29. 06. 2018, 1 female, 6 males, 29. 06. - 13. 07. 2018, 1 female. Frequent. Ponto-Caspian. Hostplant unknown.

Genus *Pachyprotasis* Hartig, 1837

75. *Pachyprotasis rapae* (Linné, 1767): 20. 04. - 05. 05. 2016, 1 male, 05-20. 05. 2018, 1 female, 35 males, 20. 05. - 01. 06. 2018, 5 males, 01-15. 06. 2018, 2 males, 15-29. 06. 2018, 2 females, 29. 06. - 13. 07. 2018, 3 females, 13-27. 07. 2018, 2 females, 3 males. Common Holarctic species. Host-

plants: *Solanum tuberosum*, *Pedicularis palustris*, *Angelica sylvestris*, *Veronica beccabunga*, *Betonica officinalis*, *Corylus avellana*, *Salix caprea*, *Fraxinus excelsior*, *Tussilago farfara*, *Symphoricarpos albus*, *Scrophularia*, *Solanum*, *Solidago virgaurea*, *Verbascum*, *Origanum vulgare*, *Atropa belladonna*, *Sarothamnus*, *Senecio*, *Polygonum*, *Lamium*, *Aspidium*, *Epilobium*, *Hypericum*, *Galeopsis*, *Glechoma*, *Mentha*, *Polystichum*, *Plantago*, *Misopates*, *Veronica*, *Quercus* and *Stachys* spp.

Genus *Rhogogaster* Konow, 1884

76. *Rhogogaster* (*Rhogogaster*) *chlorosoma* (Benson, 1943): 05-20. 05. 2018, 1 female, 29. 06. - 13. 07. 2018, 1 female. Frequent. Host plants: *Salix alba*, *S. purpurea*, *Salix* spp. Palaearctic.

77. *R.* (*Rhogogaster*) *punctulata* (Klug, 1817): 01-15. 06. 2018, 1 female. Host plants: *Salix*, *Sorbus*, *Rosa*, *Betula*, *Alnus*, *Fraxinus*, *Prunus* and *Corylus* spp. Sporadic, Palaearctic species.

78. *R (Rhogogaster) scalaris* (Klug, 1817): 20. 04. - 05. 05. 2016, 1 female. Frequent, Holarctic species. Polyphagous, larva mainly on Rosaceae, such as *Agrimonia eupatoria*, *Sanguisorba minor*, *Fragaria*, *Filipendula*, *Rosa* and *Rubus* spp.; further confirmed host plants are *Ranunculus repens* and *Alnus* spp.

Genus *Sciapteryx* Stephens, 1835

79. *Sciapteryx byzantina* Benson, 1968: 20. 04. - 05. 05. 2016, 1 female, 4 males, 05-20. 05. 2018, 2 females. Sporadic, Ponto Caspian – East Mediterranean species. Host plant unknown.

Genus *Tenthredo* Linnaeus, 1758

80. *Tenthredo* (*Tenthredella*) *albopicta* Puls, 1870: 05-20. 05. 2018, 1 female, 20. 05. - 01. 06. 2018, 1 male, 01-15. 06. 2018, 1 female, 15-29. 06. 2018, 1 female. Sporadic, Ponto-Caspian species. Host plant unknown.

81. *T.* (*Tenthredella*) *livida* Linné, 1758: 15-29. 06. 2018, 2 females, 13-27. 07. 2018, 2 female, 27. 07. - 20. 08. 2018, 1 female, 07-21. 09. 2018, 1 female. Sporadic, locally frequent. Larva on *Salix* spp., *Corylus avellana*; *Epilobium* spp.; *Lonicera* spp.; *Pteridium aquilinum*; *Rosa* spp., *Sorbus aucuparia*, *Symphoricarpos albus*, *Viburnum opulus*, *Arctium lappa* and *Athyrium filix-femina*. Palaearctic.

82. *T.* (*Tenthredella*) *procera* Klug, 1817: 05-20. 05. 2018, 1 male. West Palaearctic, sporadic species. Hostplants: *Petasites* and *Symphytum* spp.

83. *Tenthredo* (*Tenthredella*) *purpurea* Puls, 1870: 20. 05. - 01. 06. 2018, 1 female, 01-15. 06. 2018, 1 female, 15-29. 06. 2018, 1 264 m 1 female. Sporadic, Ponto-Caspian. Hostplant unknown.

84. *T.* (*Elinora*) *radoszkowskii* (André, 1881): 01-15. 06. 2018, 3 females, 15-29. 06. 2018, 3 females, 2 males, 29. 06. - 13. 07. 2018, 1 female, 07-21. 09. 2018, 1 female. Sporadic, Ponto Caspian – Anatolian species. Host plant unknown.

85. *T.* (*Tenthredo*) *scrophulariae* Linné, 1758: 29. 06. - 13. 07. 2018, 1 female. Sporadic. Larva on *Scrophularia* spp., *Buddleja alternifolia*, *Buddleja davidi* and *Verbascum* spp. West Palaearctic.

86. *T.* (*Paratenthredo*) *shaposhnikovi* (Dovnar-Zapolskij, 1930): 20. 05. - 01. 06. 2018, 1 female, 01-15. 06. 2018, 3 females, 1 male, 13-27. 07. 2018, 1 female. Sporadic, Ponto-Caspian species. Hostplant unknown.

87. *T.* (*Paratenthredo*) *talyshensis* Zhelochovtsev, 1988: 20. 04. - 05. 05. 2016, 11 females, 9 males, 05-20. 05. 2018, 9 females, 20. 05. - 01. 06. 2018, 1 female, 1 male. Sporadic, Ponto Caspian – East Mediterranean – Persian species. Host plants: *Paeonia lactiflora*, *P.* × *suffruticosa*, *P. daurica*, *P. lutea* and *P. peregrina*.

Genus *Tenthredopsis* Costa, 1859

88. *Tenthredopsis litterata* (Geoffroy, 1785): 01-15. 06. 2018, 6 males, 15-29. 06. 2018, 5 females, 19 males, 13-27. 07. 2018, 1 female. (Local colour variation It was originally described as *T. ligata* Knw. Its special genitalia structures (penial valve and hypopygium, completely agree with *T. litterata* Geoffr., although, their colour patterns are completely different fron the Central European specimens). Frequent. Larva on *Agrostis*, *Dactylis* and *Calamagrostis* spp. West Palaearctic.

89. *T. viridis* Zhelochovtsev, 1941: 05-20. 05. 2018, 8 males, 01-15. 06. 2018, 2 females. Sporadic, Ponto-Caspian species. Host plant unknown.

Discussion

Species richness

Eighty-nine sawfly species were identified from a total of 2482 specimens collected in Kintrishi National Park at 1264 m atitude by Malaise traps. Twenty species are new records for the fauna of Georgia. Six genera *Periclista* Konow, 1886, *Amauronematus* Konow, 1890, *Eupontania* Zinovjev, 1985, *Euura* Newman, 1837, *Phyllocolpa* Benson, 1960 and *Pontania* Costa, 1852. This brings the number of Sympyta species recorded in Georgia to 250. We believe that this number will at least double in our future studies.

Frequent species

The most frequent species is *Allantus cinctus* (Linné, 1758) with 604 specimens. Other frequent species are *Empria longicornis* (Thomson, 1871) with 228 and *Athalia lugens* (Klug, 1815) with 210 specimens. These 3 species make 42% of the total collected material.

Table 1. Zoogeographic distribution of the collected sawfly material

Zoogeographical area	Number of species	%
Ponto-Caspian-Persian	1	1.1%
Ponto-Caspian	7	7.9%
Ponto-Caspian-Anatolian	1	1.1%
Ponto-Caspian-East Mediterranean	1	1.1%
Persian - East Mediterranean	1	1.1%
Ponto-Caspian-Turanian	1	1.1%
West-Palaearctic	28	31.5%
Palaearctic	29	32.7%
Southern Palaearctic	1	1.1%
Holarctic	17	19.1%
Cosmopolitan	2	2.2%

The zoogeographic origin of the collected sawflies was evaluated in Table 1. Most of the species have wide geographic distribution, i.e. Holarctic, Palaearctic, West Palaearctic, South Palaearctic, Cosmopolitan; their proportion is 87%. The so-called characteristic components are the species with limited distribution areas: Ponto-Caspian, Ponto-Caspian-Turanian, Ponto-Caspian-Central Asian. These species are: *Onycholyda trigaria* (Konow, 1897), *Birka catellata* (Konow, 1900), *Strongylogaster caucasica* Schaposchnikov, 1885, *Eutomostethus ephippium* ssp. *vopiscus* (Konow, 1899), *Macrophya hamata* ssp. *caucasicola* Muche, 1969, *Sciapteryx byzantina* Benson, 1968, *Tenthredo albopicta* Puls, 1870, *Tenthredo purpurea* Puls, 1870, *Tenthredo radoszkowskii* (André, 1881), *Tenthredo shaposhnikovi* (Dovnar-Zapolskij, 1930), *Tenthredo talyshensis* Zhelochovtsev, 1988 and *Tenthredopsis viridis* Zhelochovtsev, 1941. Their proportion is 13%. Similar proportions (13%) were experienced one year before in the other regions of the Caucasus [20,21].

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Fig 1. *Colchis mixed forest close to the Malaise trap*

Fig 2: *Landscape nest to the Malaise trap*

Fig 3: Malaise trap at 1 264 m at Kintrishi Nature Reserve

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Optimizing of nutrient media for Penicillium candidum 5-1 to increase the biosynthesis of casein-specific protease

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A B S T R A C T

This study aimed to optimize the nutrient media of the protease-producing strain *Penicillium candidum 5-1,* the strain was isolated from spoiled dairy products. The nutrient medium closest to the natural substrate was selected. Accordingly, the percentage composition of lactose, casein, and potassium dihydrogen phosphate was determined. Optimal conditions for deep cultivation, including temperature, pH, and cultivation time, were established. The results showed that by optimizing the components of the nutrient media and determining the optimal conditions, the protease activity of the strain increased approximately by 50% (0.37 U/MG).

The effect of the enzyme preparation on milk showed that the protease preparation curdles the milk instead of coagulating.

This fact indicates the presence of a different type of protease, which is synthesized in nutrient media close to natural conditions, this enzyme may be a peripheral, auxiliary (minor) component, and requires further research.

Key words: protease, microscopic fungi, extracellular protease, natural conditions, nutrient media.

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INTRODUCTION

Proteases are a very large and complex group of enzymes that are widely used in various industries. Proteases make up about 60% of the enzymes sold in the world [1, 2]. They differ in properties such as substrate specificity, active site and catalytic mechanism, temperature and pH optima. It should be noted that microorganisms of different taxonomic groups have the ability to synthesize proteases. Various species of microscopic fungi are known from which milk coagulating proteases are secreted (*Talaromyces leycettanus, Rhizomucor miehei, R. pusillus, Endothia parasitica, Mucor circinelloides, Aspergillus oryzae, Amylomyces rouxii, Rhizopus microsporus, Mucor mucedo, Mucor pusillu, Mucor racemosus, and Iprex lactis*) [3-11].

Microscopic fungi have the ability to produce extracellular proteolytic enzymes. By establishing optimum cultivation conditions of microscopic fungi, it is possible to obtain proteolytic enzymes preparation with high protease activity.

MATERIALS AND METHODS

The vitality and potential of a microorganism to produce enzymes intensively much depends upon the selection of the appropriate nutrient media, in particular, carbon, nitrogen, phosphorus sources; For in-depth cultivation of active protease-producing strain *Penicillium candidum-5-1*, the following nutrients were used: lactose - 2%, casein - 2%, KH2PO4 -0.2 %, MgSO4 - 0.1%; Deep cultivation of individual strains was carried out in 250 ml Erlenmeyer conical

flasks on temperature-controlled rotary shaker (180-200 rpm), at 30°C for 72-96 hours. 10-day conidia culture suspension served as the cultivation material.

To determine the optimal concentrations of the selected carbon, nitrogen, and phosphorus sources, various percentage compositions of each source were used. The nitrogen source, casein, was tested at the following concentrations: 0.5%, 1%, 1.5%, 2%, and 2.5%. The carbon source, lactose, was taken at the following concentrations: 1%, 2%, 3%, 4%, and 5%. The phosphorus source, Potassium dihydrogen phosphate, was used at the following concentrations: 0.05%, 0.1%, 0.5%, and 1%.

Protease activity was determined in the culture fluid by Sigma's method [12].

Temperature is the most important factor for detecting the growth and physiological activity of microorganisms, first of all, the influence of temperature on the activity of the enzyme produced by the selected strain was studied. *Penicillium candidum-5-1* was cultivated in the temperature range of 25-40^0 C, with a temperature interval of 5^{0}C.

Also, the physiological activity of microorganisms is greatly influenced by nutrient media pH. The strain *Penicillium candidum-5-1* was cultivated at pH in the range from 5.0 to 9.0 with an interval value of 0.5 in the starting nutrient medium.

To study the duration of deep cultivation, the strain *Penicillium candidum-5-1* was grown in a selected liquid medium (pH 6.5) at 30^{0}C (taking into account the optimal conditions of the strain). In order to reveal the dynamics of protease accumulation in a nutrient medium, the producer of this enzyme was grown for 8 days, and the activity of this enzyme was determined every second day.

To obtain the technical preparation, cooled alcohol (-15°C) was slowly added to the supernatant at a ratio of 1:4. The solution was kept in the refrigerator at 2°C for 4 hours. Centrifugation was performed at 6000 rpm for 20 minutes to obtain the precipitate. The resulting enzyme preparation was then dried using a freez dryer, and the enzyme activity was measured.

For the milk coagulation activity assay (MCA), 0.5 mL of enzyme solution was added to 5 mL of skim milk in a 12 mL test tube pre-incubated at 35 °C for 10 min. The skim milk solution consisted of 10 g dry skim milk/100 mL of 0.01 M CaCl2. The clotting time was quantified in Soxhlet units (SU) according to the following formula: SU=(2400∗5∗D)/(T∗0.5) where T is the clotting time (s) and D is the crude enzyme dilution. One SU is expressed as the amount of enzyme required to coagulate 1 mL of a solution containing 0.1 g skim milk powder and 0.01 M calcium chloride at 35 °C for 40 min[13].

RESULTS AND DISCUSSION

The optimization of liquid nutrient media for *Penicillium candidum-5-1* was an important stage. In addition to the type of carbon source, its concentration was also found to be important for increasing proteolytic activity. The highest enzyme activity was observed when the nutrient medium contained 4% lactose. (Fig. 1).

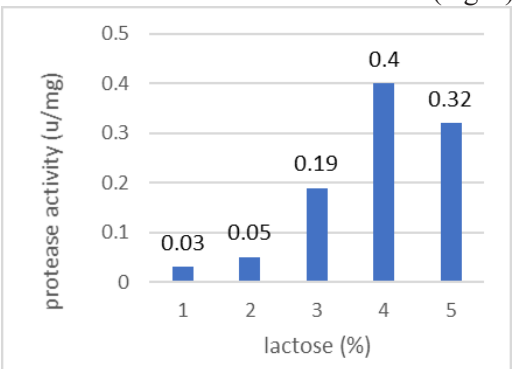

Fig.1. *Effect of different concentration lactose on protease activity Strain-Penicillium candidum- 5-1*

When different percentages of casein were added to the nutrient medium, the highest proteolytic activity was achieved at a concentration of 1.5% casein (Fig. 2).

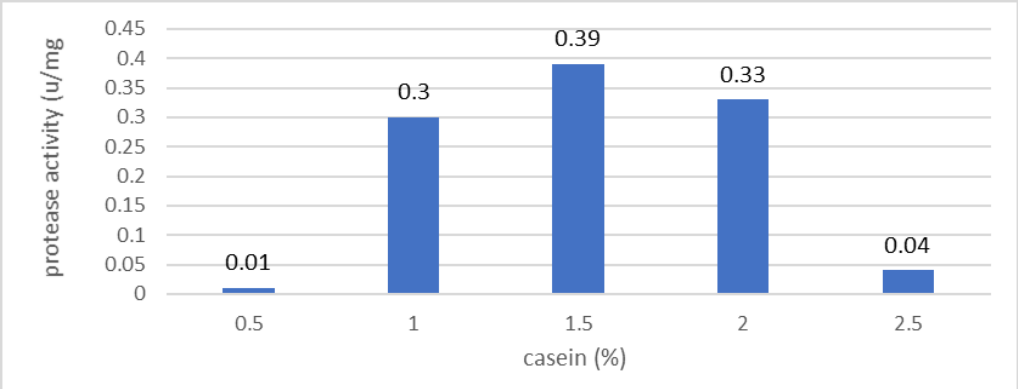

Fig. 2. *Effect of different concentration casein on protease activity Strain-Penicillium candidum- 5-1*

For Potassium dihydrogen phosphate, the highest enzyme activity was observed at a concentration of 0.1% in the nutrient medium (Fig. 3).

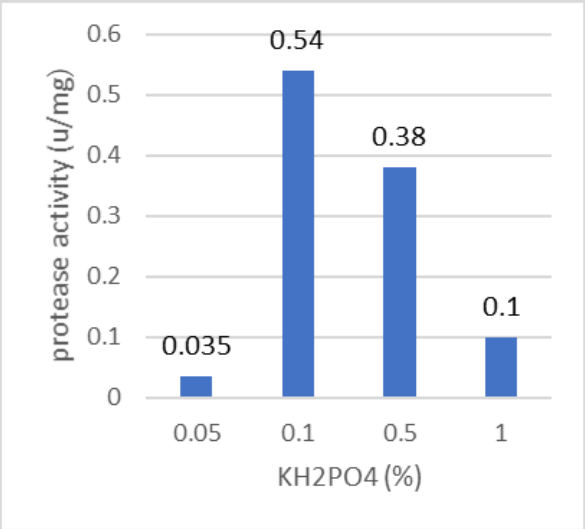

Fig. 3. *Effect of different concentration KH-2PO4 on protease activity Strain-Penicillium candidum-5-1*

The optimal temperature for protease production by *Penicillium candidum 5-1* was determined to be 30°C, at which the maximum amount of protease was produced (Fig. 4).

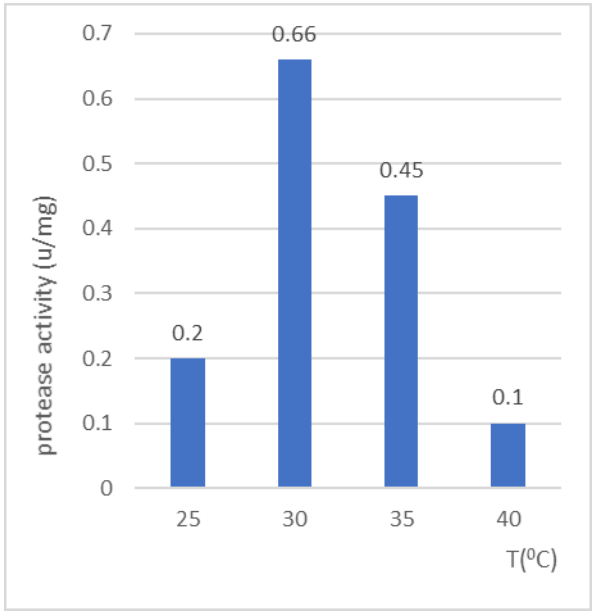

Fig. 4 *Influence of cultivation temperature on protease biosynthesis Strain-Penicillium candidum- 5-1*

The optimum pH for the cultivation of *Penicillium candidum 5-1* was found to be 6.5 (Fig. 5).

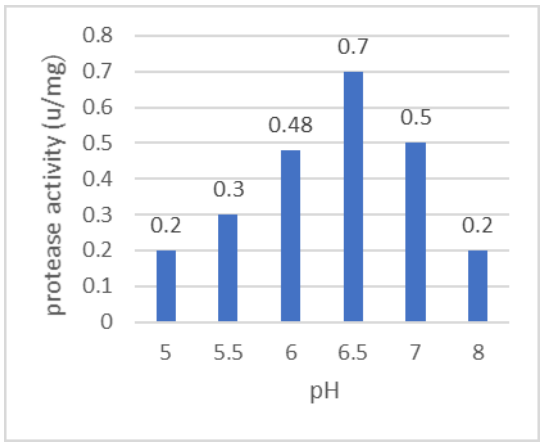

Fig. 5 *Effect of medium pH on the formation of protease Strain-Penicillium candidum- 5-1*

According to the obtained results, the production of extracellular protease started 24 hours after *Penicillium candidum-5-1* strain cultivation, and the highest activity was reached on the 6th day (144 hours). After that, a decrease in proteolytic activity was observed (Fig. 6).

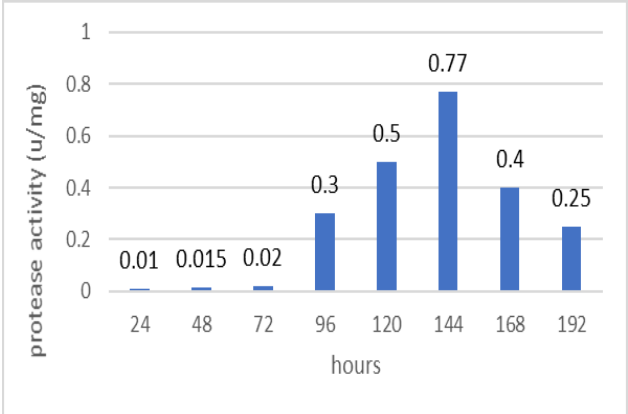

Fig. 6 *Effect of cultivation duration on the action of protease Strain-Penicillium candidum- 5-1*

As a result of the optimization of the nutrient media, the activity of protease increased by 0.37 U/MG and 50%, respectively.

Analysis of milk coagulation activity (MCA) was performed on the commercial enzyme - chymosin as a control and on the enzyme preparation obtained from *Penicillium candidum 5-1*. As a result, it was determined that the obtained enzyme preparation has a different effect from chymosin, it can curdle milk instead of coagulate (Table 1).

Table 1. *Milk-clotting activity of different concentrations of enzymes.*

Enzymes *U/MG*	Dilution factor protease	Clotting time (s)	*SU*
182	1	300	80
90,9	2	1200	40
45,4	4	4800	20
23	8	-	-
11	16	-	-
470	Commercial enzyme (chymosin) 1	300	80

This fact indicates the presence of a different type of protease, which is synthesized in nutrient media close to natural conditions, this enzyme may be a peripheral (minor) component, which is characterized by a different mechanism of action on casein and requires research.

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Study of proteins with protease activity from some plants groving in Georgia and preparation of highly active preparations

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A B S T R A C T

The purpose of this paper was to identify proteins with protease activity from some young plants in Georgia and to study them in order to obtain highly active preparations. According to the methodology developed in "Biology", several of them were selected for further work.

Key words: Vegetative organs of plants, protease activity, extraction, EWMP

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Introduction

Interest in proteolytic enzymes is great. Peptidohydrolases are characterized by selective action: they cleave only specific bonds for them. For example, pepsin acts only on the bonds established between aromatic amino acids, trypsin removes the bond between arginine and lysine, etc. The spectrum of use of proteases is very wide: in the production of dairy products - making cottage cheese, cheese, in the production of meat and meat products - softening of meat, in perfumery - bioactive additive in ointments, toothpastes, lotions; In the production of synthetic detergents - washing additive for proteinaceous contaminants, in medicine - treatment of burns, thrombosis, inflammatory processes, etc. [1-6].

From this point of view, there is a sustainable trend of interest in products of plant origin. The flora of Georgia is distinguished by the wealth of medicinal and edible plants, most of them attract attention with their chemical composition.[7-8]

Based on the above, we aimed to obtain proteins with protease activity from the raw materials selected by us for their further use.

Materials and Methods

Materials: for research Necessary reagents Na_2HPO_4, KH_2PO_4 purchased Alfa Chemical (India), Folin & Chiocalteu 's phenolic reagent and EWMP substrate "Biologica" LLC were used, Chimoral was used as a commercial protease (**Manufacturer:** Gelenica A. d. Serbia, Belgrade). The following plants were taken for research: Rubia and yellow bedstraw (Rubiaceae), yellow nanny and licorice (Fabaceae), rosebay willowherb (Onagraceae); Thyme (Lamiaceae) ; Isabella (Vitaceae). It should be noted, that all of these plants are wild, except Isabella.The Isabella grape is a cultivar derived from the grape species *Vitis labrusca* or 'fox grape,' which is used for table, juice and wine production.

Preparation of samples:

As material we took vegetative organs of plants (root, stem, leaf) in a dry state. We crushed the material, for protein extraction we added 0.1M phosphate buffer (PBS pH=7.4) at a ratio of 1/10 (mass/volume).

Determination of enzyme-substrate interaction:

The method for determining the protease activity of the enzyme is based on the effect of the enzyme on the insoluble substrate: 3 ml of 0.1 M phosphate buffer (PBS pH=7.4) and 30 µl of the enzyme solution were added to the EWMP substrate (30 mg/ml) and incubated at 27^0 C for 20 minutes . After the delay time, the substrate conversion process was stopped by placing the samples on an ice bath. After that, we centrifuged at 1500 rpm for 10 minutes.[9]. For analysis, we took the supernatant, Na_2CO_3 and so-called Folin's reagent in a ratio of 2: 5: 1. We delayed for 30 minutes. And we measured the light absorption rate spectrophotometrically (λ=660 nm). [10-11].As a control for the samples, we used the substrate solution without the enzyme, which was similarly delayed for 30 minutes, then we added the enzyme and measured immediately. The control solution prepared in this way excludes the influence of the enzyme and the substrate on absorption.

Determination of enzyme activity in various biological extracts:

To determine enzyme activity in units, units/mL and units/mg, we used the following formulas:

$$A(U)=\frac{[PR]\mu m*[Vt]ml}{[Rt]min*[Vm]ml}$$

$$A(U/ml)=\frac{[PR]\mu m*[Vt]ml}{[Rt]min*[Vm]ml*[VE]ml}=A(U)/[V_E]_{ml}$$

$$A(U/mg)=\frac{A(\frac{U}{ml})}{E(\frac{mg}{ml})}$$

$[PR]\mu ml$ Product in micromoles

$[Vt]ml$ Reaction total volume in milliliters

$[Rt]min-$ Reaction time in minutes

$[Vm]ml$ Measurement volume of the cuvette in milliliters

$[V_E]_{ml}$ Volume of Enzyme in milliliters

E(mg/ml)-Enzyme(mg.ml)

Results:

During incubation with the substrate, the proteases present in the extract begin to break down the substrate - soluble proteins and peptide fragments appear in the solution. Accordingly, the light absorption index changes. The degree of dilution depended on the rate of light absorption and the degree of its change during incubation with the substrate. We made the dilution so,that the light absorption of the extract and the substrate-enzyme complex was minimal at the initial stage, and at equal intervals of time - reflecting the kinetics of activity. We judged the intensity of the reaction by the difference between the maximum and minimum absorbance values. The proteolytic conversion of the substrate was indicated by the change in the absorbance index. According to the protein calibration curve measured by the Bradford method, we have calculated the coefficient that connects the absorption rate and the amount of protein in mg/ml (OD 1.52-0.75mg/ml). Enzyme activity was calculated as the increase in protein concentration in mg/mL/s, which indicated the accumulation of reaction product over time. The results of the study are presented in the table below:

Plants	Protein mg/ml	µg /ml/sec	U/mg
Yellow nanny	0.0064	0.26	40.63
Yellow bedstraw	0.043	1.74	40.47
Liquorice	0.049	1.54	31.43
Rosebay willowherb	0.077	1.1424	14.84
Isabella	0.127	0.17	1.34
Rubia	0.156	0.13	0.83
Thyme	0.086	0.0175	0.20

Conclusion

It can be seen from the table that the total protease activity is high in field needle, barberry and licorice, as for Isabella, Endros and Begkondara, their rate is relatively low, which does not exclude the fact that there may be proteases in these plants, the activity of which at pH≠7.4 is more low (alkaline proteases) or high (acidic proteases). This prompts us to obtain extracts from them with different extraction buffers. Based on all of the above, the biochemical research of enzymes obtained from plants with high protease activity for their further use is not devoid of interest.

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Campylobacter coli and *Campylobacter jejuni* in Georgian Retail Chicken: Isolation, Identification and Antibiotic Susceptibility

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A B S T R A C T

Campylobacter species have been presently recognized as the most frequent cause of enteric infections worldwide. To date, the prevalence and significance of *Campylobacter* infections in Georgia have not been assessed. This study aims to partially address this circumstance and provide some information on the prevalence of *Campylobacter* species in retail chicken meats sold in Tbilisi supermarkets. A total of 200 chicken meats were purchased during a two-year period. The purchased meat samples represented 6 different Georgian chicken meat producers. After isolation, 74 samples (37 %) were found to be harboring either *Campylobacter jejuni* or *Campylobacter coli,* as confirmed by MALDI TOF mass spectrometry. The isolated *C. jejuni* and *C. coli* strains were tested for antibiotic susceptibility by the disc diffusion method. *C. jejuni* and *C. coli* demonstrated high resistance to several types of antibiotics, such as ampicillin (28 % and 51 %, respectively), ciprofloxacin (79 % and 97 %, respectively) and tetracycline (28 % and 51 %, respectively).

This study concludes that 37 % of Georgian chicken meat harbors *Campylobacter* species. Most certainly, the real rate of contamination of chicken meat with these microorganisms is much higher, due to the difficulty of their isolation. Phenotypically, the local *C. jejuni* isolates differ from those of *C. jejuni* ATCC 33560.

Further studies are needed to show the clonality of the *Campylobacter* isolates, as well as their association with the diarrheal disease among patients diagnosed with enteric infections in Georgia.

Key words: *Campylobacter jejuni*, *Campylobacter coli*, Georgian Retail Chicken.

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Introduction

Campylobacter spp. are short and motile, curved microaerobic Gram-negative rods common to many different animal hosts including livestock, wild animals and pets (Battersby et al., 2016). In humans, *Campylobacter* infections are strongly associated with the consumption of contaminated chicken meat, although there are other ways of acquiring *Campylobacter* infections (Kinana et al., 2007). Out of 39 *Campylobacter* species identified to date, the most frequently isolated and clinically important species are *C. jejuni* and *C. coli,* which share 86.6 % of the genome (Kinana et al. 2007).

Infections due to *Campylobacter* spp. have been on the rise worldwide during the past two decades, along with the resistance of *Campylobacter* spp. to various antibiotics (Agunos et al., 2014). Occurrence of campylobacter-related enterocolitis in the world amounts to 400-500 million cases yearly (Vlieghe et al., 2008). It has also been estimated that worldwide 50 % of chicken meat is contaminated with *Campylobacter* spp. (Vandeputte et al., 2019).

Poultry has been recognized as the major reservoir of *Campylobacter* spp. (Connerton et al., 2018), (Hakeem & Lu, 2021). Prevalence of *Campylobacter spp.* in raw farm-raised chicken meat varies from country to country, however up to 100 % of birds may harbor this pathogen at slaughter

age. In poultry, colonization with *Campylobacter* spp. is not associated with any clinical signs. These microorganisms are localized mostly in the cecum and the cloacal crypt of birds (Le et al., 2012). Various organs, such as the liver, the small intestine, and gizzard usually also contain this pathogen (Epps et al., 2013). Contamination events with *Campylobacter* spp. occur during slaughter, leading to cross-contamination of the carcasses with rates as high as 100% (Hakeem & Lu, 2021).

In the past scientists ascribed the diversity of *C. coli* and *C. jejuni* strains to their abundance in wild birds and animals. Increasing evidence, however, suggests that anthropogenic factors are the driving force in the evolution of these microorganisms (Sheppard & Maiden, 2015). Industrial farms, with extremely large numbers of broilers, opened a novel niche for *Campylobacter* spp. resulting in the emergence of *C. jejuni* lineages able to colonize multiple hosts. In parallel, expansion of particular *C. coli* lineages common to both agricultural animals and sick humans have been taking place along with the emergence and proliferation of resistant lineages of *C. jejuni* and *C. coli* with genetic exchange between these lineages (Sheppard & Maiden, 2015).

Although most of human campylobacteriosis cases are self-limiting, complications may occur as post-infection sequelae (Yang et al., 2019). Seriously debilitating post-infection complications include Guillain Barre syndrome, Reiter's syndrome and Irritable Bowel Syndrome (IBS). Additionally, children, the elderly and immunocompromised patients may suffer complications resulting from *Campylobacter* infections (Facciolà et al., 2017).

In most developing countries, costly reagents as well as difficulties of culturing *Campylobacter* spp. make the isolation and identification of these microorganisms challenging (Ghorbanalizadgan et al., 2019). Often identification of *Campylobacter* spp. is not a part of the routine laboratory workup. For these reasons, many cases of human campylobacteriosis in developing countries are frequently misdiagnosed.

An equally important issue is the uncontrolled use of antibiotics and antimicrobial substances in the meat and other agriculture industries (Serwecińska, 2020). Whether the particular poultry farms involved in this study engage in the use of antibiotics for sub-therapeutic purposes, could not be confirmed. However, judging from the high levels of resistance observed against penicillin, fluoroquinolones and tetracyclines among the isolated *Campylobacter* spp., it may be concluded that birds are frequently exposed to these substances in local poultry farms (Yang et al., 2019).

Materials and Methods

Sample Collection

Samples were collected over a two-year period, from fall 2018 to fall 2020. During this time chicken carcasses, chicken livers, chicken breasts and thighs from various meat producers were purchased in Tbilisi supermarkets. The samples, as packaged, were placed in clean plastic bags and kept in a cooler bag until needed. Usually, the purchased samples were processed for the isolation of *Campylobacter* spp. within 2-3 hours from purchase. One separate chicken carcass or a single package of chicken livers were treated as one isolation instance. Overall, about 200 samples of 6 different producers were purchased resulting in 74 isolates. Seasonality was not taken as a factor in this study.

Isolation and Culturing of *Campylobacter*

Sampling

Chicken breasts and thighs were sampled with sterile cotton swabs. Chicken carcasses were sampled in multiple areas. The swabs were then placed for enrichment into sterile 15 mL conical tubes containing 5 mL of buffered peptone water. At the same time, parts of the skin (about 15 g of the neck, wing, and thigh or rump area) were removed and placed for enrichment into a sterile 50 mL conical tube containing 25 mL of buffered peptone water. Chicken livers were placed in a sterile petri dish and minced with a sterile lancet, after which the minced portion of the liver was transferred into a 15 mL tube with peptone water for the enrichment step.

Enrichment Media and Conditions

Samples were enriched using buffered peptone water (pH 7.0), (Biolife, Italy). The enrichment time did not exceed 2 hours. The incubation temperatures of 37°C and 42°C were tested prior to conducting isolations of *Campylobacter* spp. (data not shown). The optimal growth temperature for isolation of *Campylobacter* spp. was determined to be 37°C (data not shown).

Culture Media

Dehydrated Cefoperazone Charcoal Deoxicholate Agar/mCCDA/Campylobacter Blood Free Medium Base, Bolton (Biolife, Italy) was used for inoculating the enriched samples. Campylobacter Selective Supplement (Liophilchem, Spain) containing cefoperazone (16 mg/L) and amphotericin B (5 mg/L) was added into the medium after cooling off to 50°C, according to the manufacturer's instructions (Liofilchem, Italy). Samples were inoculated at 50 uL per plate using the four-quadrant isolation method. Sample inoculation on mCCDA agar plates was performed in quadruplicates, to increase the chance of isolation.

Culturing Conditions

37°C and 42°C temperatures were tested for culturing *Campylobacters*. Candles and anaerobic jars were used to create microaerobic atmosphere. It was determined that 37°C worked better for isolation of *Campylobacter* spp. 42°C temperature was used for subculturing isolated strains.

Identification of *Campylobacter* spp.

Staining:

Initially, Gram's staining kit (Deltalab, Italy) was used and then a single 1 % carbol fuchsin (Deltalab, Italy) stain to rapidly identify presumptive colonies of *Campylobacter* spp.

Confirmation by Campylobacter Latex Agglutination Test

Catalase activity and Campylobacter Latex Agglutination Test (Liofilchem, Italy) were used to confirm suspect colonies as *Campylobacter* spp. *C. jejuni* ATCC 33560 strain served as positive control.

Subculturing

Colonies that morphologically and microscopically resembled those of *Campylobacter* spp. were carefully picked with the blunt end of a sterile 10 µL inoculation loop, transferred onto the first quadrant of the CCDA agar plate and then inoculated on four quadrants. The inoculation loop was sterilized in between each quadrant. Each strain was subcultured from a discreet colony until pure culture was obtained (usually 2-3 times).

MALDI-TOF Mass Spectrometry

Campylobacter species were identified by Mass Spectrometry performed on Matrix Assisted Laser Desorption Ionization Time of Flight mass spectrometer (MALDI-TOF MS; Vitek-MS, Biomérieux, Nürtingen, Germany) at the Institute for Medical Microbiology and Hospital Hygiene of the University Hospital, Magdeburg.

Briefly, bacterial cultures were plated on Columbia blood agar (Oxoid) and incubated under microaerophilic conditions. After 48 hours, a single bacterial colony from a monoculture was used for identification. For this purpose, a colony was touched very lightly with the tip of a sterile toothpick and spread upon predefined spot on a barcoded slide with 48 spots, which were first pre-treated with 5 µL of the matrix solution. After all samples were transferred on the slide, it was run on the Mass-Spectrometer.

Antibiotic Susceptibility Testing

Kirby Bauer disk diffusion method was used to determine antibiotic susceptibility of the *Campylobacter* spp. Testing was performed on all confirmed *Campylobacter* isolates and interpreted according to the guidelines provided by 2022 European Committee on Antimicrobial Susceptibility (EUCAST).

Antibiotic disks (Oxoid) were placed on Mueller Hinton Agar plates supplemented with 5 % sheep's blood and inoculated with 0.5 McFarland standard of each respective bacterial strain monoculture. The susceptibility plates were incubated for 48 h at 37°C under microaerophilic conditions, after which the inhibition zones were measured. The zone diameters were interpreted as susceptible (S), or resistant (R) after the EUCAST guidelines (Paintsil et al., 2021).

Isolates resistant to at least one antibiotic from each of the following antimicrobial groups - tetracyclines, macrolides, and quinolones - were considered multi-drug resistant (MDR), which is defined as resistance to three or more antimicrobials of any substance group.

Table 1. *Breakpoints for Determination of Antibiotic Resistance of Campylobacter Isolates - C. coli and C. jejuni**

Antibiotic (disk concentration)	Zone Diameter (mm)	
	S≥	R<
Tetracycline (30 µg)	30	30
Ciprofloxacin (5 µg)	50	26
Erythromycin (15 µg) *C. coli*	20	20
Erythromycin (15 µg) *C. jejuni*	24	24
Ampicillin (10 µg)	13*	7*
Chloramphenicol (30 µg)	18*	18*
Kanamycin (30 µg)	15*	7*
Streptomycin (25 µg)	22*	13*

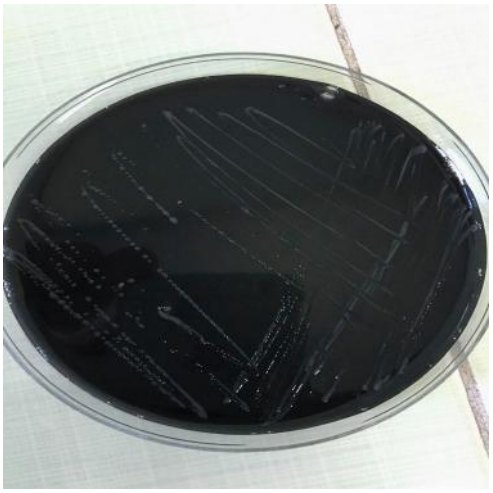

Figure 1. *Campylobacter spp. Isolate on a CCDA Plate*

Catalase Test

All suspect colonies displayed somewhat delayed catalase activity. Data not shown.

Latex Agglutination Test

The latex agglutination test kit was intended to identify *C. jejuni* isolates. This test was used at the initial stage to confirm the suspect colonies and it was observed that all suspect colonies resulted in agglutination, as compared to the positive control. Data not shown.

*Speciation of the **Campylobacter** Isolates using MALDI TOF Mass Spectrometry*

By using Mass Spectrometry analysis, 74 isolates that were presumed to belong to *Campylobacter* spp. were identified as *C. jejuni (*n=39) and *C. coli* (n=35).

Results

Colony Appearance

On CCDA agar *Campylobacter jejuni* colonies were observed as large, off-white, droplet-like or irregular-shaped and elongated, mucoid colonies. *Campylobacter coli* colonies were often observed as grayish-brown, oval-shaped or rounded, discrete, medium- to- smaller sized colonies. However, intermediate looking colonies were often observed as well.

Microscopy

Microscopy of the isolated strains revealed fine, pleomorphic, gram-negative rods. Staining of the local *Campylobacter* isolates did not reveal the classic "seagull wing" shape. Rather, the stained bacteria appeared either having a somewhat elongated and serpentine shape, or having a shorter, comma-like or "S"-shape appearance.

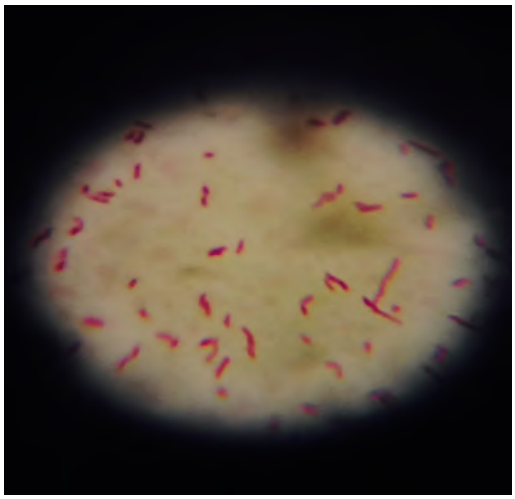

Figure 2. *Campylobacter spp. Isolate Stained with Carbol Fuchsin*

Table 2. *Speciation of Campylobacter Isolates using MALDI TOF Mass Spectrometry*

ID-ed Species	N of Isolates	Prevalence
C. jejuni	39 isolates	19.5 %
C. coli	35 isolates	17.5 %

Antibiotic Susceptibility Testing

Among the 35 isolates of *C. coli*, the highest resistance was observed to ciprofloxacin (97. 14 %), ampicillin (51.43 %), and tetracycline (51.43 %). One strain (CC- 20) was also resistant to both streptomycin and chloramphenicol (2.86 %).

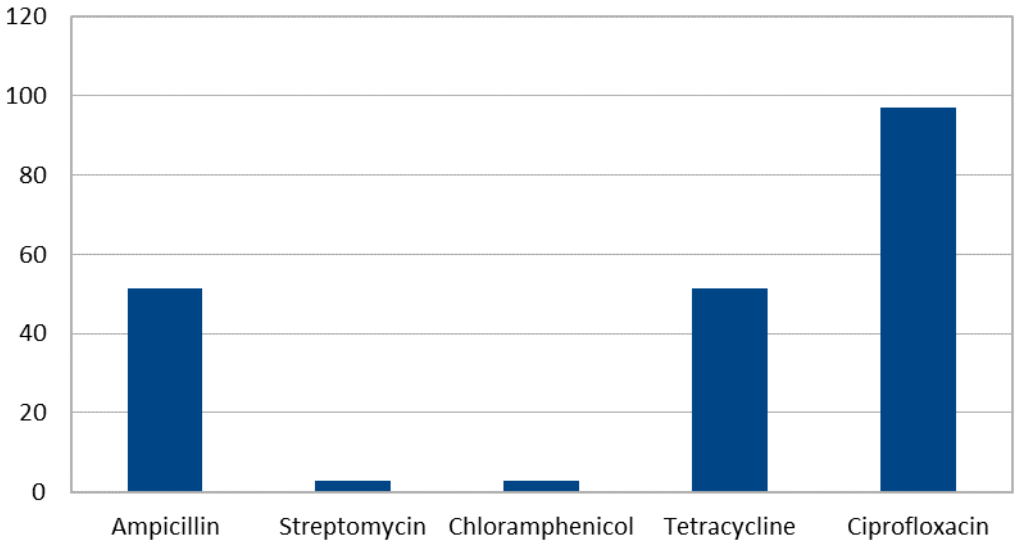

Figure 4. % Resistance of *C. jejuni* Isolates to Various Antibiotics

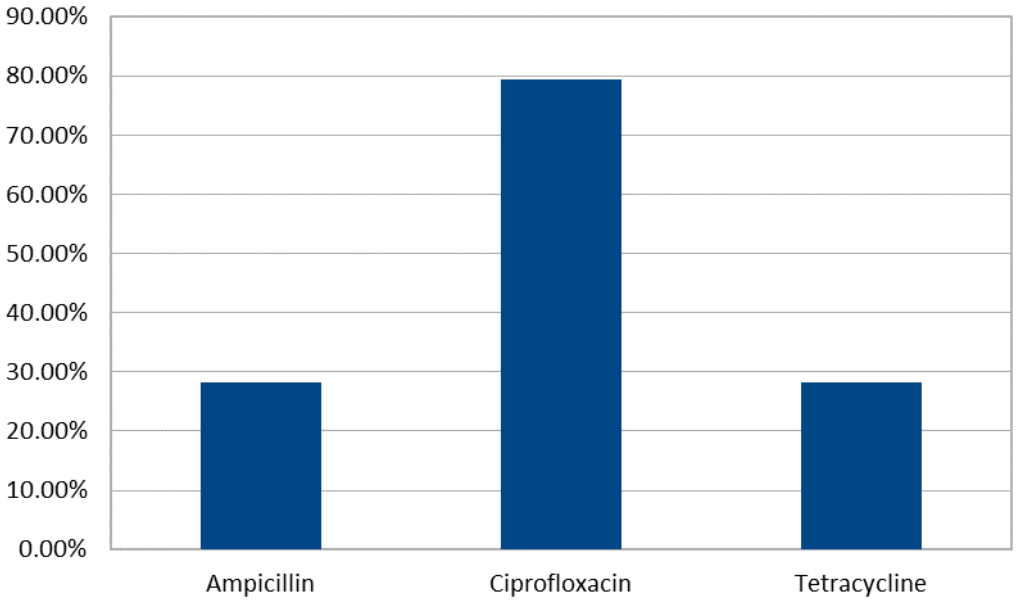

Figure 3. % Resistance of *C. coli* Isolates to Various Antibiotics

Resistance of the *C. jejuni* isolates to the same antibiotics appeared somewhat lower, however still at alarmingly high percentages: 28 % of the isolates were resistant to ampicillin and tetracycline, while 79 % showed resistance to ciprofloxacin.

As a result, 60 % of *C. coli* isolates and 28 % of *C. jejuni* isolates demonstrated resistance to at least three different classes of antibiotics, and thus qualifying as MDR (multi drug resistant) strains.

Discussion

This study is the first example of isolation of *Campylobacter* spp. from Georgian retail chicken meats. Various published methods, notably that of Oyarzabal et al., recommend mCCDA agar and CCDA antibiotic supplement containing cefoperazone and amphotericin B, for isolation of *Campylobacter* spp. (Oyarzabal et al., 2013).

The abundance of co-contaminant bacterial species made isolation of *Campylobacter* challenging in this study. Firstly, the antibiotic supplement did not inhibit many Gram-positive and some Gram-negative bacterial species. Secondly, allowing 48 h enrichment time resulted in the inhibition of slower-growing *Campylobacter* spp. by the faster-growing co-contaminant bacteria that also thrived in microaerobic conditions and 42°C. Thus, to limit the unwanted growth of irrelevant species, 37°C was used as the enrichment temperature, while the enrichment time was cut down to 2 hours.

Since Bolton broth also caused exuberant growth of irrelevant bacteria, sample enrichment was performed using buffered peptone water. It was essential that meat samples were completely submerged in the liquid. Vortexing samples several times during the enrichment step and prior to inoculation was also important to dislodge the bacteria from meat. 50 μL of sample was inoculated onto four different mCCDA agar plates after incubation.

Microscopy required some optimization as well. Initially, every suspect colony was stained using the Gram's method. Due to the fact that safranin stains *Campylobacter* poorly, resulting in bad microscopic visualization of the sample, we used another, fast direct staining method described by Mushi et al. for quickly identifying *Campylobacter* colonies. As a result, smears of suspect colonies were stained for 30 sec with 1 % carbol fuchsin and immediately examined for the presence of curved or pleomorphic rods (Mushi et al. 2014). The positive colonies were subcultured until pure culture was obtained. This technique saved a lot of time during the process of screening bacterial colonies.

Biochemically, *Campylobacter* spp. are relatively inert and they poorly hydrolyze sugars. To distinguish between species, scientists rely on a few biochemical characteristics (Burnett et al., 2002). One such biochemical test is identification of *C. jejuni* by its ability to hydrolyze hippurate. However, about 10% of *C. jejuni* isolates are incapable of hydrolyzing hippurate. Moreover, there is another, hippuricase-positive species-*C. avium,* first isolated in Italy in 2006 from chicken (Miller et al., 2017). To avoid confusing results, for species identification purposes we used MALDI TOF mass spectrometry. This method relies on databases of previously run and validated profiles of microorganisms and delivers results that are almost 100 % accurate. University of Magdeburg's Laboratory of medical Microbiology and Hygiene kindly provided us with this service.

Proportions of the poultry isolates of *C. jejuni* and *C. coli* among *Campylobacter* spp. isolated in a specific country vary geographically. In Canada, for example, *C. jejuni* dramatically outnumbered *C. coli* among *Campylobacter* spp. isolated from chicken: 87 % vs. 12 % (Dramé et al., 2020). In a Brazilian study, all 87 % of the total *Campylobacter* isolates turned out to be *C. jejuni* exclusively (Rodrigues et al., 2021). In other countries *C. coli* was isolated at the rates equal to or exceeding those of *C. jejuni*. For example, a recent Australian study found that the majority of *Campylobacter* isolates from fresh and frozen chicken carcasses and meats were those of *C. coli* (50-77%), whereas *C. jejuni* isolates were more common (50-88%) in beef, pork and lamb (Walker et al., 2019). A 2020 Chinese study identified *C. jejuni* and *C. coli* at almost equal numbers (233 and 231, respectively) among the 464 isolates of *Campylobacter* spp (Tang et al., 2020). A 2016 Italian study identified more *C. coli* than *C. jejuni* among their campylobacter isolates (Pergola et al., 2017). A higher percentage (75.5 %) of *C. coli* was also identified among the *Campylobacter* isolates from chicken meat compared to *C. jejuni* (24.5 %) in a 2011-2013 Polish study (Szczepanska et al., 2017). However, another Polish study concluded that *C. jejuni*, not *C. coli,* was the predominant *Campylobacter* species in Polish poultry meats (Szosland-Fałtyn et al. 2018). A similar 2015 study from Thailand also identified more *C. coli* (n=94) than *C. jejuni* (n=36) among the isolates from samples taken in and around chicken farms and hatcheries (Thomrongsuwannakij et al., 2017) and an Argentinian study of 2011-2013 found that *C. jejuni* outnumbered *C. coli* both in kosher (36% to 2 %) and conventional (26 % to 4 %) meats. This finding agreed with previous studies conducted in Argentina where *C. jejuni* isolates significantly outnumbered those of *C. coli* (Guirin et al., 2020).

Thus, the fact that the prevalence of *C. coli* and *C. jejuni* in Georgian chicken is somewhat equal is not unusual and agrees with reports from other research groups.

Due to the widespread practice of antibiotic use in food animals in the past decades in order to prevent and control infections, or even to enhance the growth of food animals, *C. jejuni* and *C. coli* have often been reported as resistant to penicillins (Wieczorek & Osek, 2013). In this study too, all *Campylobacter* isolates were resistant to penicillin G. However, resistance to ampicillin was somewhat lower among *C. jejuni* isolates compared to C. *coli* (28 % vs. 53 %). These findings are similar to a 2011 Irish study, which detected resistance to ceftifur (58 %), ampicillin (25 %) and nalidixic acid (17 %) among *C. jejuni* strains (Madden et al., 2011).

Resistance of *Campylobacter* spp. to tetracycline is also frequently reported worldwide, since tetracycline is the most widely used antibiotic in avian production due to its low cost (Wieczorek and Osek, 2013). In this study, 51 % of *C. coli* strains and 28 % of *C. jejuni* strains were found to be resistant to tetracycline. The tet(O) gene, which causes this resistance, seems to have global presence and

has been detected in many parts of the world. For example, an Irish research group reported that 100 % of the chicken isolates of thermophilic *Campylobacters* were harboring the tet(O) gene (Lynch et al., 2020). Resistance to tetracycline was also high-98 % and 56 %-among all *Campylobacter* isolates in a 2017 study conducted in Thailand, while most isolates were also MDR strains (Thomrongsuwannakij, Blackall, and Chansiripornchai, 2017).

Resistance to ciprofloxacin was the highest among the *Campylobacter* spp. isolated in this study. Similarly, Polish scientists reported that 91 % of *C. jejuni* isolates were resistant to this antibiotic (Wieczorek and Osek, 2013). An Indian research group observed that the highest rate of resistance among the *C. jejuni* isolates from chicken meat was to nalidixic acid and ciprofloxacin - 81.25 % and 63.46 %, respectively (Sathiamoorthi, T., Joseph Sahayarayan, J. and Arivoli, A., 2016). High resistance levels to tetracycline, ciprofloxacin and nalidixic acid (79.1 %, 72.1 % and 65.1 %, respec - tively) were observed in a 2017 Italian study as well (Pedonese et al., 2017). High incidence of fluoroquinolone resistance was seen among the isolates of both species (100 % and 98.9 % in *C. jejuni* and *C. coli*, respectively) in a study conducted in Thailand.

Antibiotic resistance finds its way to wild bird populations as well. For example, tetracycline and fluoroquinolone resistant strains of *C. coli* and *C. jejuni* were identified in storks by a 2015 Polish study (Szczepańska et al., 2015).

Our findings are in agreement with reported by other scientists from around the world, indicating at the growing tendency of antibiotic resistance among *Campylobacter* spp. An extremely worrisome trend is the emergence of MDR *Campylobacter* strains. As mentioned previously, many isolates of *Campylobacter* spp. from Georgian retail chicken have shown resistance to 3 antibiotics of different classes: penicillins, tetracyclines and fluoroquinolones. One *C. coli* isolate (CC-20) showed additional resistance to chloramphenicol (a macrolide drug) and streptomycin (an aminoglycoside drug).

Conclusion

Georgian retail chicken harbors at least two *Campylobacter* species, *C. jejuni* and *C.* coli, with high percentage of MDR strains among them. High resistance to Ciprofloxacin and Tetracycline point to circulation of these antibiotics in local farms. Previous scientific opinion that the majority (90 %) of the food-borne illnesses are associated with *C. jejuni,* may no longer be valid. Prevalence of certain *Campylobacter* spp. in types of meats and in human and animal hosts clearly varies geographically (Igwaran & Okoh, 2019). Therefore, it remains to be ascertained which *Campylobacter* species are associated more frequently with human campylobacter infections in Georgia.

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List of species of Pentatomids (Hemiptera: Pentatomidae) in the hazelnut orchards of Sakartvelo (Georgia) and their potential parasitoids

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A B S T R A C T

During this survey in the hazelnut orchards of Sakartvelo (Georgia) 12 species of pentatomids were recorded. The following species are recorded from Georgia: *Graphosoma lineatum* (Linnaeus,1758), *Halyomorpha halys* (Stal,1855), *Pentatoma rufipes* (Linnaeus,1758), *Rhaphigaster nebulosi* (Poda,1761) and *Nezara viridula* (Linnaeus,1758). From the 72 recorded species in Georgia 21 of them have parasitoids from the superfamilies Platygastroidea and Chalcidoidea and this number reaches 32 species from Palaearctic region. However only 14 species of them had been recorded from Caucasus and 9 from Georgia, where hosts are known. Additionally, *Trissolcus manteroi* (Kieffer, 1909) and *Tr. oobius* (Kozlov, 1972) from the Caucasus are reported, but their hosts are unknown.

Key words: Brown Marmorated Stink Bug, *Trissolcus, Ooencyrtus, Anastatus*

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Introduction

The stink bugs in the family Pentatomidae represents one of the most species diverse group among insects, icluding around 5000 species worldwide [1]. They can be serious economic pests of food crops and ornamental plants around the world [2,3]. Many pentatomids cause significant damage to hazelnut crops in Europe, Turkey and Georgia [4], however there are predators too. According Tavella et al. [5], hazelnut in northwestern Italy was damaged by seven species of true bugs: two coreids, one acanthosomatid and four pentatomids. The Brown Marmorated Stink Bug - *Halyomorpha halys* (Stål) (BMSB) (Hemiptera: Pentatomidae), which is native to East Asia (China, Taiwan, Japan, and South Korea), is a serious threat to agriculture in North America and Europe due to its polyphagous behavior and absence of natural enemies in its invaded range [6]. In its native range, BMSB causes extensive damage to various fruits and soybeans, and it has recently become a serious pest of apples in Japan [7].

BMSB is known to overwinter inside houses and other enclosed structures, becoming a nuisance pest for homeowners before migrating in the spring into crops and weedy areas where its population develops large numbers during summer and fall [6,8,9].

It must be noted that much research has focused on assessment of biological control agents available in its native range of Asia that offer effective population suppression, specifically parasitoid wasps [10,11]. However, exploration for alternative local natural enemies in it's newly invaded areas is actively going on [12,13,14].

The list of hemipterans distributed in Georgia (=Sakartvelo) was done by Zaitseva [15,16] and included 72 species. The list of pentatomid parasit-

oids distributed in Georgia have not been reported previously.

Material and methods

The sampling was conducted in hazelnut orchards of five different regions of Sakartvelo: Guria, Achara, Samegrelo (Western Georgia), Kartli and Kakheti (Eastern Georgia). Eight field trips were organized during the 2019 growing season, from April to November. Beating trays were used for collecting BMSB individuals. The egg masses collected from the field were taken to the laboratory, and rearing was conducted in cages. Species of stink bug collected during the surveys were identified by the first author. *Trissolcus* spp. were treated by Japoshvili et al. [14].

As the most species of *Trissolcus* are olygophagous, and they can parasitize alternative hosts, the literature sources were surveyed to make list of pentatomid parasitoids distributed in the Palaearctic, Caucasus and Georgia, to know potential of biocontrol. All species recorded (reared) for these pests from throughout the Palaearctic region are listed. The species distributed in Caucasus are marked with one asterisk and species from Georgia with two asterisks (Table 1). Parasitoid list was completed based on Kozlov, Kononova [17], Talamas et al. [18], Noyes [19], Powell [20] and Japoshvili et al. [14].

It should be noted that many of the *Trissolcus* parasitoids were identified prior to the taxonomic treatments by Talamas et al. [18] and Tortorici et al. [21], and these publication provide the basis for our name usage. We consider it most likely that identifications of *Tr. flavipes* (Thomson) were actually *Tr. cultratus* (Mayr) [22] and treat these as such in our list.

Results

List of pentatomids recorded in Georgian hazelnut orchards:

Pentatomidae

Genus *Acanthosoma* Curtis, 1824

1. *Acanthosoma haemorrhoidale* (Linnaeus,1758)

Material examined: Georgia, Gonio (1), 12.05.2019

Genus *Carpocoris* Kolenati, 1846

2. *Carpocoris fuscispinus* (Boheman,1850)

Material examined: Georgia, Dziguri.(4), 10.04.2019; Zakagori(1), 6.07.2019.

3. *Carpocoris pudicus* (Poda,1761)

Material examined: Georgia, Shakriani (2), 17.07.2019; 18.06.19; Khobi (1), 11.07.2019.

Genus *Dolycoris* Mulsant & Rey, 1866

4. *Dolycoris baccarum* (Linnaeus,1758)

Material examined: Georgia, Dziguri sasaplao (15), 10.04.2019; Mta Peria (3), 14.04.2019. Shakriani (1), 10.05.2019.

Genus *Elasmucha* Stal 1864

5. *Elasmucha betulae* (De Geer,1773)

Material examined: Georgia, Eniseli (1), 18.06.2019.

Genus *Eurydema* Laporte de Castelnau, 1833

6. *Eurydema oleracea* (Linnaeus,1758)

Material examined: Georgia, Shakriani (1), 17.05.2019.

7. *Eurydema ornata* (Linnaeus,1758)

Material examined: Geoegia, Shakriani (4), 10.05.2019.

Genus *Graphosoma* de Laporte, 1832

8. *Graphosoma lineatum* (Linnaeus,1758)

Material examined: Georgia, Shakriani, 18.06.2020.

Genus *Halymorpha* Mayr, 1864

9. *Halymorpha halys* (Stal,1855)

Material examined: Georgia, Junetseri (3), 15.07.2019; Khobi (3), 11.07.2019; Agruni (1), 13.10.2019; Lanchkhuti (1), 12.04.2019.

Genus *Mustha* Amyot & Serville, 1843

10. *Mustha spinosula* (Lefebvre,1831)

Material examined: Georgia, Isani (1), 26.06.2019.

Genus *Pentatoma* (Olivier, 1789)

11. *Pentatoma rufipes* (Linnaeus,1758)

Material examined: Georgia, Tsitelmta (1), 15.06.2019.

Genus *Rhaphigaster* Laporte, 1833

12. *Rhaphigaster nebulosa* (Poda,1761)

Material examined: Georgia, Eniseli (1), 18.06.2019.

Note: Number of individuals are given in brackets.

Conclusion

Our survey in the hazelnut orchards of Sakartvelo (Georgia) revealed 12 species of pentatomids, among them are well known pests of different agricultural and forest plants: *Graphosoma lineatum*, *Halyomorpha halys*, *Pentatoma rufipes* and *Rhaphigaster nebulosa*. Literature sources records also *Nezara viridula* [16], which was not found during our

studies in Hazelnut orchards. From the 72 recorded species in Georgia [16] 21 of them have parasitoids from Platygastroid and Chalcidoid families and this number reaches 32 species from the Palaearctic region. However only 14 species of them had been recorded from Caucasus and 9 from Georgia, where hosts are known. Additionally, *Tr. manteroi* and *Tr. oobius* for Caucasus are reported, but their hosts are unknown.

This data gives very optimistic hopes in the biological controlling measures against such dangerous pests as it is Brown Marmorated Stink Bug – *Halyomorpha halys*. Thus future intensive investigations are needed to elaborate effective integrated pest menegment methods and reduce insecticide mass applications.

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Table 1. The pentatomids (Hemiptera: Pentatomidae) species recorded from Sakartvelo with their parasitoids recorded from the Palaearctic region.

№	**Pentatomidae**	**Parasitoids**
1	*Aelia rostrarta* Boh	Platygastroidea
		Trissolcus colemani (Crawford)** *Tr. rufiventris* (Mayr)* *Telenomus chloropus* (Thomson)**
		Chalcidoidea
		Ooencyrtus pityocampae (Mercet) *O. telenomicida* (Vassiliev)**
2	*Aelia acuminata* L.	Platygastroidea
		Trissolcus basalis (Wollaston) *Tr. belenus* (Walker)** *Tr. colemani* (Crawford)** *Tr. semistriatus* (Nees)** *Tr. scutellaris* (Thomson)** *Tr. rufiventris* (Mayr)*
		Chalcidoidea
		Ooencyrtus nigerrimus Ferriere & Voegele *O. pityocampae* (Mercet) *O. telenomicida* (Vassiliev)**

3	*Arma custos* F.	Platygastroidea
		Telenomus heydeni Mayr*
4	*Apodiphus amygdali* Germ	Platygastroidea
		Trissolcus saakowi (Mayr)* *Tr. tumidus* (Mayr)**
		Chalcidoidea
		Oopristus erganicus Tarla & Doganlar *O. tayfursokmeni* Tarla & Doganlar *O. turkestanicus* Skriptshinsky
5	*Carpocoris fuscispinus* Boh	Platygastroidea
		Trissolcus basalis (Wollaston) *Tr. rufiventris* (Mayr)* *Tr. scutellaris* (Thomson)** *Telenomus chloropus* (Thomson)**
		Chalcidoidea
		Ooencyrtus nigerrimus Ferriere & Voegele *O. pityocampae* (Mercet) *O. telenomicida* (Vassiliev)**
6	*Carpocoris pudicus* Poda	Platygastroidea
		Trissolcus colemani (Crawford)** *Tr. flavipes* (Thomson)* *Tr. scutellaris* (Thomson)** *Tr. viktorovi* Kozlov**
		Chalcidoidea
		Ooencyrtus nigerrimus *O. pityocampae* (Mercet) *O. telenomicida* (Vassiliev)**
7	Dolycoris baccarum L.	Platygastroidea
		Trissolcus belenus (Walker)** *Tr. colemani* (Crawford)** *Tr. mitsukuri* (Ashmead) *Tr. rufiventris* (Mayr)* *Tr. scutellaris* (Thomson)** *Tr. semistriatus* (Nees)** *Tr. viktorovi* Kozlov** *Telenomus chloropus* (Thomson)** *Te. heydeni* Mayr*
		Chalcidoidea
		Ooencyrtus pityocampae (Mercet) *O. telenomicida* (Vassiliev)** *O. solidis* Khlopunov

8	*Eysarcoris ventralis* Westw	Platygastroidea
		Telenomus chloropus (Thomson)**
9	*Eurydaema oleracea* L.	Platygastroidea
		Tr. belenus (Walker)** *Tr. colemani* (Crawford)**
10	*Eurydema ornata* L.	Platygastroidea
		Trissolcus basalis (Wollaston) *Tr. belenus* (Walker)** *Tr. colemani* (Crawford)** *Tr. scutellaris* (Thomson)* *Tr. semistriatus* (Nees)** *Tr. viktorovi* Kozlov**
		Chalcidoidea
		Ooencyrtus telenomicida (Vassiliev)** *Anastatus bifasciatus* (Goeffroy)
11	*Eurydema spectabilis* Hrv	*Trissolcus viktorovi* Kozlov**
12	*Eurydema ventralis* Kol.	*Trissolcus scutellaris* (Thomson)** *Tr. colemani* (Crowford)** *Tr. belenus* (Walker)**
13	*Graphosoma lineatum* L.	Platygastroidea
		Trissolcus belenus (Walker)** *Tr. colemani* (Crawford)** *Tr. rufiventris* (Mayr)* *Tr. scutellaris* (Thomson)** *Tr. semistriatus* (Nees)** *Tr. viktorovi* Kozlov** *Telenomus chloropus* (Thomson)**
		Chalcidodiea
		Ooencyrtus gonoceri Viggiani *O. nigerrimus* Ferriere & Voegele *O. pityocampae* (Mercet) *O. telenomicida* (Vassiliev)**
14	*Graphosoma semipunctatum* F.	Platygastroidea
		Trissolcus basalis (Wollaston) *Tr. belenus* (Walker)** *Tr. bennisi* (Voegele) *Tr. colemani* (Crawford)** *Tr. scutellaris* (Thomson)** *Tr. semistriatus* (Nees)**
		Chalcidodiea
		Ooencyrtus gonoceri Viggiani *O. nigerrimus* Ferriere & Voegele *O. pityocampae* (Mercet) *O. telenomicida* (Vassiliev)**

15	*Halyomorpha halys* Stal	Platygastroidea
		Trissolcus cultratus (Mayr)** *Tr. japonicus* (Ashmead)
		Chalcidodiea
		Anastatus bifasciatus (Goeffroy)** *Ooencyrtus telenomicida* (Vassiliev)*
16	*Holcostethus venalis* Wolff.	Platygastoirea
		Trissolcus belenus (Walker)** *Tr. colemani* (Crawford)**
17	*Neottiglossa pusilla* Gmel.	*Trissolcus colemani* (Crawford)**
18	*Nezara viridala* L.	Platygastroidea
		Trissolcus basalis (Wollaston) *Tr. mitsukurti* Ashmead *Tr. scutellaris* (Thomson)**
		Chalcidoidea
		Ooencyrtus nezarae Ishii *Ooencyrtus nigerrimus* Ferriere & Voegele *Ooencyrtus papilionis* Ashmead *Ooencyrtus pityocampae* (Mercet) *Ooencyrtus telenomicida* (Vassiliev)** *Anastatus bifasciatus* (Geoffroy)** *Anastatus japonicus* (Ashmead) *Acroclisoides* sp. *Pteromalus* sp.
19	*Palomena prasina* L.	Platygastroidea
		Trissolcus belenus (Walker)** *Tr. cultratus* (Mayr)** *Tr. elasmuchae* (Watanabe) *Telenomus chloropus* (Thomson)** *Te. heydeni* Mayr*
		Chalcidoidea
		Anastatus japonicas (Geoffroy)** *Oencyrtus telenomicida* (Vassiliev)**
20	*Palomena viridissima* Poda	Platygastroidea
		Telenomus chloropus (Thomson)** *Te. heydeni* Mayr*
21	*Pentatoma rufipes* L.	Platygastroidea
		Trissolcus kozlovi Rjachovsky *Tr. scutellaris* (Thomson)**
22	*Rhaphigaster nebulosa* Poda	Platygastroidea
		Telenomus heydeni Mayr*
		Chalcidodiea
		Ooencyrtus pityocampae (Mercet) *Anastatus bifasciatus* (Goeffroy)**