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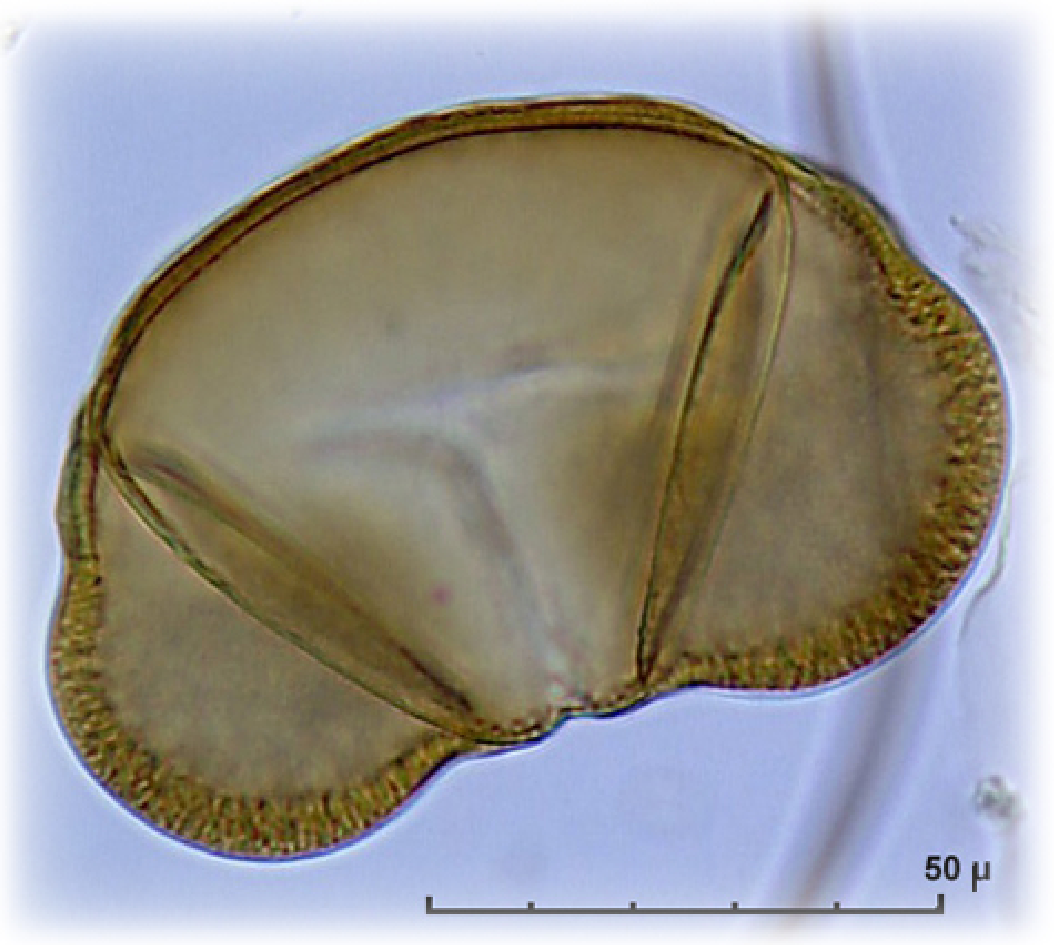

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REGULAR ARTICLES

SHORT COMMUNICATION

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Original picture on front cover: *Picea abies* fossil pollen,
in equatorial view © Ioan Tanțău

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Assessing the performance of *Alphitobius piceus* (Oliver, 1792) as novel feeder insect species for small sized postmetamorphic frogs

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Abstract. One of the major difficulties of frog farming is providing adequate food in the first few weeks after the metamorphosis. This is a critical time frame, with rapid growth and high mortality. The established feeder insect species used throughout this delicate stage (cricket and mealworm larvae, adult fruit flies) are suitable from a trophic perspective, but present challenges and difficulties that influence the production costs. The aim of this study is to assess the performance of an alternative feeder species – *Alphitobius piceus* – with a simpler production technology and a lower production cost. Two species of feeder insect were used: the conventionally used *Acheta domestica* larvae as control species, and *Alphitobius piceus* larvae as experimental species. As model anuran we used the European common frog, *Rana temporaria*. The experimental insect species was easily accepted and produced a strong feeding response in the post-metamophic frogs. There were no significant differences between the control and experimental groups in terms of body condition index and mortality, however survival was better in the experimental group. This similarity indicates a high potential of *Alphitobius piceus* as feeder species for the newly metamorphosed frogs and also a good economic opportunity for frog farms.

Keywords: Frog farming, live prey, feeding response, juvenile frogs.

Introduction

Frog legs are a popular and valuable product; therefore, frog farming becomes one of the most attractive commercial enterprise in aquaculture (Miles *et al.,* 2004). However, before considering setting up an edible frog farm, the problem of constantly supplying a large quantity of live prey must be addressed and resolved. The production of live food becomes, therefore, an activity as important as the production of the frogs themselves. Keeping the cost of this production at the lowest possible level, through a reduced infrastructure and a simple technological process, will be a top priority and a good indicator of the profitability.

Live prey size is also an important problem to address. Providing the right type of food item can be especially problematic in the period right after the metamorphosis, because of the small size of the postmetamorphic animals. This is a critical period for the frogs, when the growth rate (Breckenridge and Tester, 1961; Labanick and Schlueter, 1976) and the mortality (Pechmann *et al.*, 1991; Roznik and Johnson, 2009; Scott *et al.*, 2007) is the highest, and they are not yet able to feed on live maggots. At this stage, the animals are commonly fed on adult common fruit flies (*Drosophila melanogaster*), cricket larvae in early stages (*Grillus* sp. *Acheta* sp.) or early-stage larvae of the darkling beetle (*Tenebrio molitor*) (Miles *et al.*, 2004; Culley *et al.*, 2009). All of these feeder species satisfy the trophic needs of the frogs but their production and handling can often prove problematic. For example, cricket farming technology is complex and needs a significant infrastructure (Hanboonsong *et al.*, 2013), the darkling beetle is easy to grow and reproduce, but separating the early instar stage larvae needs a fairly complicated tray stack rearing system (Morales-Ramos, 2012) and the common fruit fly has a high escape capacity. These are apparently small inconveniences but they translate in to higher production costs. Therefore, the identification of a novel, more advantageous feeder insect species for post-metamorphic frogs is a timely enterprise.

The neuro-ethological studies conducted by Ewert (1980) have largely deciphered the nature of the stimuli that trigger feeding behaviour in anurans. Thus, the most appreciated prey shape proved to be the most worm-like, similar to a horizontal line with a strong contrast to the substrate.

In this study we propose to bridge the gap between metamorphosis and maggot eating size frogs, using *Alphitobiul piceus* (the lesser mealworm) – a small sized beetle – as a possible candidate. The species is easy to grow and reproduce and the larvae seem to be suitable as shape (Ewert, 1980) and size (Kuzmin, 1990) (Fig. 2), making it potentially more useful than the species generally used as food in the early post-metamorphic stage.

The purpose of the study is rearing juvenile post-metamorphic frogs in a more cost-effective way by testing the efficiency of the lesser mealworm larvae as experimental food source for post-metamorphic juveniles of *Rana temporaria*, compared with the control feeder insect species *Acheta domesticus*. For a better perspective on the potential advantage of the experimental species, we focused on the resultant growth and mortality rather than the consumption rate of the food items.

Materials and methods

The model anuran

We used the European common frog (*Rana temporaria*) as experiment species. It is a semiaquatic edible species, widely consumed ethno-gastronomicaly throughout Transylvania (Lengyel, 2016) and has a LEAST CONCERN status in the IUCN Red List of Threatened Species. At adult stage, it's food consists of a wide spectrum of terrestrial invertebrates, arthropods and mollusks, the differences of diet preferences between populations being attributed only to the availability of the prey species (Tiberti *et al.*, 2015). Consequently, the common frog behaves like a generalist predator, eating all mobile prey types large enough to be observed and small enough to be swallowed. Although it has a semiaquatic ecology, the feeding behaviour of the postmetamorphic juveniles is similar to that of many aquatic species including *Lithobates catesbeianus,* which often leaves the safety of the water, especially during night-time activity, and feeds mainly on terrestrial insects (Viosca, 1931). Also, the common frog has been previously used in other aquaculture related feeding studies (Miles *et al.*, 2004).

Three entire Common frog clutches were collected on February 25, 2018, from three different temporary ponds located in the Faget forest Cluj-Napoca, Cluj county, Transylvania, Romania (46°41'48,57''N 23°32'46,80''E (DMS), Someș-River Basin, elevation 682 m). The egg clutches were kept separately, in three 10 litre aquariums containing dechlorinated tap water. The hatching period lasted for 6 days (03. 27. 2018 - 04. 02. 2018). At Gosner stage 25 (Gosner, 1960) we randomly selected 20 healthy-looking larvae from each clutch (a total of 60 tadpoles). The remaining tadpoles were released in the original ponds.

The selected larvae were raised in 4L opaque containers (9x17x26 cm) at a density on 2,5 individuals / litre, corresponding to low densities in natural populations (Glennemeier and Denver, 2002; Rot-Nikcevic *et al.*, 2005). The water temperature was kept constant at 20 ° C ± 1 ° C at a circadian rhythm of 12/12 hours of light / dark. The light was switched on and off at 9am and 9pm respectively and was provided by four 36W neon tubes. Each container had an independent oxygen supply through electric pumps. Roughly 90% of the water was replaced daily with dechlorinated tap water. The food residues were cleaned permanently, by siphoning.

The diet of the larvae was predominantly of vegetable origin: spirulina (Organic spirulina 500 mg, protein 63.5%, carbohydrates 16.1%, lipids 8.2%. Origin: China), pelleted rabbit feed (Versele Laga Cuni fit pure, protein 15 %, carbohydrates 15% lipids 3% Origins: Hungary) and lettuce (3% protein, 6% carbohydrates, 1% lipid, USDA), supplemented once a week with food of animal origin (lyophilized tubifex, Bio-lio, protein 54 %, lipid 16%, origin: Hungary) (Petranka and Kennedy, 1999).

The metamorphosis period lasted for 7 days (04.26.2018 - 05.02.2018). With the emergence of the forelimbs (ca. Gosner 42), the water quantity was reduced and one end of the container was raised so that the metamorphs can climb out. The metamorphosed frogs were relocated in containers specific to the terrestrial environment.

The postmetamorphic juveniles were held in specially designed opaque plastic containers (27x17x9cm), with textured side walls (Ewert *et al.*, 2004), at a density of 4 animals / container. For the substrate, a wash-cloth-like material with high moisture retention capacity was used. A shelter was provided in the form of two branches of artificial plants with leaves (Exoterra forest plant) in order to mimic the natural conditions (Craioveanu *et al.*, 2017) and also a terracotta pipe (Ewert *et al.*, 2004). In the case of the experimental group, on the opposite side of the shelter we placed a petri dish (Ø 90 mm) as food recipient (Fig. 1).

At the end of the experiment, all surviving animals were released in the original location.

The feeder insect species

Alphitobius piceus (Coleoptera; Tenebrionidae) is a small-sized beetle species (Fig. 2), from the family *Tenebrionidae*, known commonly as the lesser mealworm. In our study, we used the larvae as experimental food.

After hatching, the larva is ca. 1mm long and reaches a maximum of 12 mm before developing into a pupa. The colour is dark yellow to light brown, with variations in intensity, offering a good contrast with both dark and light colour substrates. It feeds on a wide range of plant and animal origin (e.g. dead adults or their own exuviae) foods. The number of instars is variable (9-20) and the pupa stage lasts for 5-30 days, depending on environmental factors. The adults are 5-8mm long, dark brown to glossy black and live ca. 30 days.

The species is a prolific breeder. The female lays about 500 eggs, directly in the substrate. Depending on the temperature and humidity of the environment, the hatching time varies between 5 and 20 days. The sexes are monomorphic.

Lesser mealworms are generally considered cereal pests and are adaptable to a wide range of environmental factors with a very high tolerance threshold. Due to these characteristics, the species is well suited for intensive growth.

In the absence of the species' specific growth protocols and information, and due to the ecological and phylogenetic similarity with the flour beetle (*Tenebrio molitor*), we used as a guide the growth technology developed for the latter.

The growth substrate was prepared according to Klasing *et al*. (2000), with a content of 75% wheat bran (protein 15%, SC Agromar SRL, Romania) and 25% chicken starter feed (Breco, Covasna, Romania). The environmental temperature was 28°C ± 1°C (Fraenkel, 1950) and the environmental humidity approx. 40% with a 24/24 hours lighting consisting of two 36W neon tubes.

The growing containers (60cm L x 30cm l x 20cm h) were half filled with the substrate mixture and seeded with approximately 200 adults. As a water source we used fruits (e.g. a quarter of an apple/week/container). Covering the containers was not necessary because the species does not have the ability to climb vertical surfaces or to fly. The first harvest was made after 60 days. The larvae harvesting method is simple and is done by placing Barber traps, directly in the substrate with pieces of fruit or vegetables used as bait.

Acheta domestiucs (Orthoptera; Gryllidae) is an invasive orthopteran species with an adult body length of 16-21mm and a light brown colour. In this study, the species was used as a control food. The life cycle lasts 8-12 weeks from egg, through 8 larval stages, to adult. The two sexes resemble each-other except for the ovipositor of the female. It is a prolific breeder, lays roughly 50-100 eggs in the substrate with moderate humidity, at a depth of 6-25mm. The hatching period takes about 2 weeks. As adult, the male produces a characteristic stridulating sound.

Farming technology: Adults used for breeding were kept in glass aquariums (60cm L x 30cm l x 35cm h) at a density of ca. 1 cricket / 10 cm^2 (Clifford *et al.*, 1976). To increase the active surface and reduce stress, the bottom of the aquarium was almost completely covered with egg cartons (Nakagaki and Defoliant, 1991). Water was provided by a wash-cloth soaked in water and placed in a petri dish. The ambient temperature was maintained at 28°C ± 1°C, the humidity at about 40% and the lighting was on 24/24 (2 x 36W neon tubes) (Clifford *et al.*, 1976).

Food was provided once a day and consisted of a ground mixture (granules of max 1 mm, mesh 150) of granulated feed for rabbits (Versele-Laga, Belgium, Protein 14.0%, Fat 3%, Crude cellulose 20%, Crude ash 7%, Calcium 0.6%, Phosphorus 0.4%), wheat bran (SC Agromar SRL, Romania, Balotești, protein 15%) and starter feed for chicken (Breco, Romania, Covasna). In addition to the dry food, we also provided various fresh plants (clover, alfalfa, dandelion) (Nakagaki and Defoliant, 1991; Clifford *et al.*, 1976).

For reproduction, we placed egg-laying plastic containers in the adult enclosures (14cm L x 9cm L x 5cm h) filled with high humidity substrate (80-90%). In order to achieve an aerated consistency, the substrate was obtained by mixing potting soil (Agro CS Universal Substrate, Brasov, Romania) with medium-grained sand (Desert Sand, black, Exoterra, USA), in 50:50 ratios. The mere presence of these egg-laying boxes in the breeding colony intensifies the breeding behaviour and triggers ovipositor activity in females. To prevent the cannibalization of eggs by the adults, the boxes were covered with a 2mm wire mesh.

The containers were kept in the breeding colony for 3 days, after which they were replaced with new, unused ones. The seeded containers were moved in plastic boxes (27x17x9cm) equipped with a lid, to maintain humidity. The first larvae hatched after ca. 13 days. Given the high humidity needed for moulting, especially in the early larval stages, the newly hatched larvae were raised in the hatching container until reaching larval stage IV.

We estimated the appropriate size of the prey as the distance between the eyes of the frog. As a consequence, only crickets in larval stage I, II and III were used as feeder for the first two months of the post-metamorphic juveniles' lives.

In order to determine the nutritional value of the two food types used in the experiment, we performed a raw chemical composition analysis (Tab. 1) using Weende's system of analysis.

The chemical analysis was performed at the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania, and the feeder insects used in this study were produced in the Vivarium of the Babes-Bolyai Univerity, Cluj-Napoca, Romania.

The study was conducted in accord with the highest humane and ethical principles, according to the ARRIVE guidelines for In Vivo Experiments.

The experimental design

To test the effectiveness of the experimental feeder insect as food for the postmetamorphic juveniles of the common frog, two groups were used: an experimental group, fed with *Alphitobius piceus* larvae and a control group, fed with *Acheta domesticus* larvae. Each group consisted of 20 postmetamorphic frogs (n=20/group) and was divided into 5 replications (4 animals/replication). We set the size of the 2 groups to n=20 according to previous studies on dietary diversification in frog farms (Miles *et al.*, 2004). The experiment lasted for 60 days. At the end of the experimental period, all the surviving animals have reached the necessary maggot eating size.

Measurements and analysis

In order to monitor the evolution of the two groups, we measured and recorded the following variables:

1. Snout to urostyle length, in mm (SUL)

To avoid the excessive handling of animals, a digital imaging technique was used. The retention method was described by Antwis and Browne (Antwis and Browne, 2008, on amphibianark.org), and consists of placing the specimen in a Petri dish and covering it with a lid, in order to obtain a more horizontal position. Also, the thickness of the Petri dish has to be approximately equal to the thickness of the animal. Following this procedure, images were taken of each individual with a Nikon D 3200 digital camera, mounted at a distance of 30 cm above the specimens. The photos were subsequently analysed and measurements were performed using ImageJ open source image processing software (http://imagej.nih.gov/ij).

2. Mass

After the photographs were taken, the animals were dried by placing them briefly on filter paper and weighed with an analytical electronic balance (0.01 g accuracy).

3. Mortality in %

During the 60 days of the experiment (the estimated critical post-metamorphosis time frame), three sets of measurements were performed, on day 0 of the experiment (16.05.2018), on day 30 of the experiment (18.06.2018) and on day 60 (17.07.2018).

The body condition index of the animals was calculated (BCI), according to the residual linear regression model (Băncilă *et al.* 2010), for each treatment (group fed with *Achaeta domesticus* and group fed with *Alphitobius piceus*), and for each measurement session (May 16, June 18 and July 17, 2018). Before performing this analysis, to ensure that the values of SUL and mass meet the criteria for linear regression analysis, for all measurements, we analysed the data as follows:

- graphical analysis using scatter graphs (scatter.smooth function in RStudio)
- graphical analysis using boxplot graphs to identify aberrant values (boxplot function in RStudio)
- graphical analysis using density graphs to highlight the normal distribution of data (density and polygon functions in RStudio)
- calculation of the correlation between SUL and body mass, for each measurement, using Pearson's or Spearman rank correlations, depending on the data distribution (the cor.test function in RStudio).

The preliminary testing of the data was followed by the elaboration of the linear regression model using the function lm (RStudio). The list of the residual values was obtained using the residuals function (RStudio).

The obtained BCI values were tested for normal distribution using the Shapiro-Wilk test and then compared between the two feeding treatments (with *A. domesticus* and *A. piceus*), for each measurement session, using Welch two sample t test.

We also calculated the mortality rate in the two feeding treatments, at each measurement session, and compared them using the Mann-Whitney U-test.

All analyses were performed with Rstudio open source software, version 1.1.463 (2016).

Results

During the three measurement sessions, the average snout to urostyle lengths and body masses were consistently higher for the group fed with crickets (Tab. 2), without the differences between the Body Condition Indexes of the two groups being statistically significant (Tab. 3).

The largest discrepancy between the mean BCI is within the second measurement, with more positive values for the *A. piceus* fed group and more negative for the *A. domesticus* fed group (Tab. 3). The distribution of BCI data (Fig. 3) shows that most values concentrate around 0, indicating a body condition very close to that estimated by the mathematical model for both treatments. There were no differences in BCI between treatments in any of the performed measurements (Tab. 4, Fig. 3).

The mortality percentages of *Rana temporaria* juvenile animals for each replication and each treatment has been summarized in Table 6.

Although the level of animal mortality was consistently higher in the cricket-fed group (Tab. 5), the two groups did not differ significantly from each other (Mann-Whitney test: $p > 0.05$ for each measurement).

Table 1. Raw chemical composition of the experimental (1) and control (2) feeder insect

Species	Dry matter	Crude protein (%) as sampled	Crude protein (%) in dry matter	Crude fat (%) as sampled	Crude fat (%) in dry matter	Crude ash (%) as sampled	Crude ash (%) in dry matter
1. *Alphitobius piceus*	31.76	18.33	57.74	8.12	25.58	1.40	4.40
2. *Acheta domesticus*	25.72	16.87	62.79	4.66	18.11	1.69	6.57

Table 2. Mean values, standard deviations and limits of the snout-urosyle length (mm) and body weight (g) during the three measurement sessions

Diet	Ms	N	$\overline{X} \pm s_x$ (Snout-urostyle length - SUL)	$\overline{X} \pm s_x$ (Body weight - BW)	Range (SUL, mm)		Range (BW, g)	
					Minimum	Maximum	Minimum	Maximum
Cricket diet	L1	20	20.51 ±1.49	0.69 ±0.12	18.2	23.1	0.4	0.9
	L2	16	26.19 ±1.97	1.61 ±0.22	21.9	29.4	1.0	2.0
	L3	10	27.69 ±1.40	2.01 ±0.20	25.0	29.8	1.8	2.4
Lesser mealworm diet	L1	20	21.04 ±1.28	0.67 ±0.11	18.1	23.7	0.5	0.9
	L2	18	25.46 ±1.79	1.50 ±0.29	22.0	28.4	1.1	2.1
	L3	12	26.99 ±1.57	2.09 ±0.37	24.0	29.1	1.7	2.8

Ms=measurement session; L1=first measurement; L2=second measurement; L3=third measurement; N=group size; $\overline{X}$=mean; s_x=standard deviation; Range=extreme values of the measurements;

Table 3. Mean values and limits for the body condition index of the animals fed with the two species of experimental food (*A. domescus* and *A. piceus*)

Measurement session	**Experimental food**	**$\overline{X}$±**	**s_x**	**Range**	
				minimum	**maximum**
L1	*A. domesticus*	$-3.4 * 10^{-07}$	±0.8786	-1.67413	1.723816
L1	*A. piceus*	$-1 * 10^{-09}$	±0.8179	-1.84637	1.234487
L2	*A. domesticus*	$-1.3 * 10^{-09}$	±1.4340	-2.00525	3.346637
L2	*A. piceus*	$1.11 * 10^{-09}$	±1.1797	-2.39603	1.556069
L3	*A. domesticus*	$-1.1 * 10^{-09}$	±0.7361	-1.20132	1.597073
L3	*A. piceus*	$-1.4 * 10^{-09}$	±1.2179	-2.46773	1.450089

L1=first measirement; L2=second measurement; L3=third measurement; $\overline{X}$=mean; s_x=standard deviation; Range=extreme values of the measurements

Table 4. Results of comparisons between body condition indices of R. temporaria animals fed with the two feeder insect species (Welch two sample t-test), in the three measurement sessions

Measurement	**Value of t**	**Degrees of freedom**	**Value of p**
16 May (L1)	$-1.272 * 10^{-06}$	37.807	1
18 June (L2)	$-5.204 * 10^{-09}$	29.164	1
17 July (L3)	$7.787 * 10^{-10}$	20.969	1

Table 5. Mean values and range of the mortality (%) of the animals for the two experimental groups for each replication and for the three measurement sessions

***Acheta* sp.**	**N**	**$\overline{X} \pm s_x$**		**Range**	
				minimum	**maximum**
Measurement 1	5	0		0	0
Measurement 2	5	20±	20.917	0	50
Measurement 3	5	55±	11.18	50	75
***Alphitobius* sp.**					
Measurement 1	5	0		0	0
Measurement 2	5	10±	13.693	0	25
Measurement 3	5	30±	20.916	0	50

Figure 1. Enclosure for postmetamorphic frogs.

Figure 2. *Alphitobius piceus*, larvae and imago.

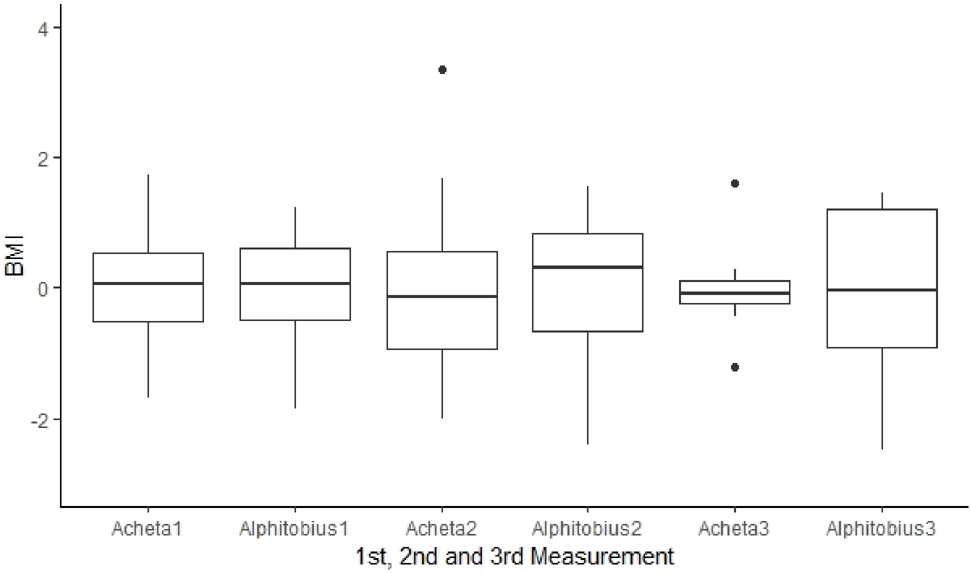

Figure 3. Values of the body condition indices (BMI) of the animals in the two different treatments (animals fed with *Acheta domesticus* and animals fed with *Alphitobius piceus*) during the three measurement sessions (1st-May 16, 2nd-June 18 and 3rd-July 17). Plots represent median (line inside boxes), 25–75 percent quartiles (boxes) and minimal and maximal values. Outliers are represented with a dot.

Discussion

Providing the right type of food and also a good management of the associated costs is a pivotal point in frog farming and represents important indicators in anticipating the success or failure of a commercial enterprise.

Although frogs are highly specialized predators, the search for novel feeder species or inert peleted food items will be a constant preoccupation of the industry and the related scientific comunity (Lima *et al*., 1986; Rodriguez-Serna *et al*., 1996; Braga *et al*., 1998; Castro *et al.,* 2001).

The aim of this study was to identify an alternative, low cost, food item for the juvenile postmetamorphic frogs in the most vulnerable period of their life cycle. We also tested if this type of food has an appropriate quality.

Considering the trophic needs of the frogs in early postmetamorphic development, the two compared diets showed a high similarity, in terms of crude protein and crude ash (Tab. 1). This similarity was indeed reflected in no significant differences between the control and the experimental group from the perspective of the body condition index (Tab. 4).

From a behavioural point of view, the two species could have elicited different feeding responses, considering the lower speed and mobility of the experimental insect. However, in our study, the experimental food was easily accepted and the specific" worm like" shape produced a strong feeding response. The results are in line with earlier neuro-ethological (Ewert, 1980) and neuro-physiological (Beauquin and Gaillard,1998) studies that indicated a strong preference of amphibians towards horizontal, slow moving, prey-like objects.

Regarding the mortality rate, although statistically insignificant, there was an important difference between the two groups (55% group fed *A. domesticus* versus 30% group fed *A. piceus*; Tab. 5, measurement no. 3). We cannot exactly identify the source of this difference, but we suppose that it comes from the method of presenting the two types of feeder insect species. Cricket larvae were released freely into the rearing containers while the lesser mealworms were offered in a Petri dish. Consequently, the cricket-fed animals had to actively hunt for prey, with probably less success, while those fed on lesser mealworms, once accustomed with the location of the food, were able to feed without significant effort.

Conclusions

An overall conclusion of this study is that the experimental feeder insect produces similar level of nutritional performances as the control feeder insect, in laboratory conditions.

In the real-world conditions of a frog farm, we believe that *Alphitobius piceus* has key characteristics to enhance productivity at a potentially lower cost and improve economic profitability.

As a delivery method, we recommend placing the larvae in shallow feeding trays, in the close proximity of the shoreline. As a result of this layout, the juvenile frogs will have good access to food, with most of the feeding occurring during night time activity.

If used for a longer period, there is a reasonable expectation that the experimental insect would be better accepted in time, considering that exposure to a particular prey strengthens the feeding behaviour response to that prey in amphibians (Jaeger and Barnard, 1981). Also, according to stomach content analysis, many frog species prey largely on adult beetles in the wild (Korschgen and Moyle, 1955; Stojanova and Mollov, 2008). The adult of *Alphitobius piceus* has a number of characteristics that could simplify its large scale production (e.g. the adult does not fly, does not have the ability to climb vertical surfaces), therefore, although it has not been the subject of this study, we consider that the adult stage also represents a good potential food item in frog farming.

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Comparative analysis of the digestive system's anatomical parts in two zoophagous bird species: White Stork (*Ciconia ciconia*) and Common Buzzard (*Buteo buteo*)

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Abstract. Aristotle in his anatomical observations is marking the beginning of functional anatomy followed by Georges Cuvier that formulate the low of the correlation of parts. According with this we can expect that the digestive system structures tend to be similar between species that consume approximately the same type of food. In this study we chose to evaluate macroscopic the digestive system of two different zoophytophagous species, with similar body size but different life behavior and feeding strategies: White Stork (*Ciconia ciconia*) and Common Buzzard (*Buteo buteo*). Data were collected after the dissection of 10 carcass (5 White Stork and 5 Common Buzzard). The digestive tract and its structures were measured and weighed. In common buzzard the beak is strong and short adapted for the laceration of the prey. In white stork the beak is long, strong, straight adapted to capture prey like a harpoon. In both species, the esophagus is located on the right side of the neck, the length is different, proportional to the neck. In common buzzard the crop is present and absent in white storks. The cuticle or koilin layer is highlighted in white stork compared to common buzzard. In common buzzard, the small intestine is reduced in size, without marked transition between the duodenum and the ileum. The white storks have a long small intestine with many loops, with no transition between the segments. In both species, the cecum is reduced in size and has a vestigial appearance; the colon is reduced in size, and extends from the level of the ileo-colic junction to the cloaca. The digestive system is adapted to a strictly carnivorous diet and the differences identified between the anatomical structures of the digestive tract of the two species are correlated with the differences of their feeding behavior.

Keywords: functional anatomy, esophagus, crop, intestine, feeding behavior

Introduction

Speaking about anatomy, Aristotle mentioned: "As among themselves, [birds] differ in their parts in respect of the more and less […] some of them have long legs, some short ones […] and similarly with the other parts. […] Another peculiarity of birds is the beak, an extraordinary appendage to the head. It is made of bone, and serves them instead of teeth and lips. […]. Birds' beaks also differ according to their different [ways] of life. Some beaks are straight, some curved; straight if they are used simply for feeding, curved if the bird eats raw meat, because a curved beak is useful for overpowering their prey. Those who spend their lives in swamps and are herbivorous have broad beaks, which are useful for digging and pulling up their food and for cropping plants" (Peck, 1983). In this way Aristotle in his anatomical observations and analysis brings the form in relation to function and opens, we believe for the first time, a path to the functional anatomy (Blits, 1999).

Starting from the idea of the relationship between form and function, Georges Cuvier, analysing the dissections performed on numerous individuals of different vertebrate, formulate the low of the correlation of parts. Cuvier said that based on an animal's skull, all the other anatomical structures of the systems that compose it can be deduced, all being are correlated with each other and with its life behaviour (Larson, 2006; Kardong, 2011).

These ideas about the relationship between form and function and the low of the correlation of parts were essential for the foundation of anatomy, comparative anatomy and evolutionary biology. However, nowadays observations can nuance these principles that underlying anatomy. Based on this, we can expect that the digestive system structures and its attached glands, tend to be similar between species that consume approximately the same type of food.

In this context we chose to evaluate macroscopic the digestive system with the related grands and structures of two different zoophytophagous species, with similar body size but different life behavior and feeding strategies: White Stork (*Ciconia ciconia*) and Common Buzzard (*Buteo buteo*).

Both species consume exclusively animal food. White Stork eats a wide variety of species, depending on availability and locality; in dry years, it may consume mostly insects and mice, in wet years mostly aquatic organism. They consume insects and larvae (especially Coleoptera and Orthoptera), amphibians (particularly adult frogs as *Rana esculenta, Rana temporaria*), tadpoles, reptiles (lizards, snakes), small mammals (*Microtus* and *Arvicola*, mole (*Talpa europaea*), young rats), less often young and eggs of ground-nesting birds, molluscs and crustaceous, fish and scorpions. Odd items include young goats, cats, weasels, domestic ducks and chickens (Cramps and Perrins, 1986).

Common Buzzard consumes small mammals (predominantly rodents *Microtus arvalis*, *M. agrestis*, *Arvicola terrestris, Clethrionomys glareolus,* hamsters (*Cricetus cricetus*), wood mouse (*Apodemus sylvaticus*), rats), rabbits (*Oryctolagus cuniculus*), young hare, moles (*Talpa europaea*), squirrels, birds, reptiles, amphibians, larger insects and earthworms. In summer, they eat mainly nestlings and just-fledged young, occasionally adults (Starlings (*Sturnus vulgaris*), thrushes (Turdidae), crows (Corvidae), finches (Fringilidae), larks (Alandidae), woodpeckers (Picidae)), reptiles (most frequently lizards (*Lacerta*) and slow-worm *Anguis fragilis*, snakes *Natrix natrix*, *Elaphe scalaris*, *Vipera),* amphibians (*Rana*, *Bufo, Pelobates, Bombina*). Cases of cannibalism were recorded (Cramp and Perrins, 1987).

The aims of our study were (i) to describe the components and topography of the digestive system of dissected individuals from the two species; (ii) to highlight the macroscopic anatomical peculiarities of the digestive system and (iii) to compare the data obtained after dissections and analyse them according to the particularities of the feeding behaviour.

Materials and methods

Data and observations were collected after the dissection of 10 carcass (5 of White Stork and 5 of Common Buzzard), obtained from the Zoological Museum of the Cultural University Heritage of UBB (attestation 40/19.01.2021). The cause of death of dissected individuals was result of accidents (electrocution, road accidents) or were euthanized due to injuries that no longer allowed them to be rehabilitated into the wild.

The dissection protocol was following Cătoi (2003). From the level of the head, the skin was incised on the lateral commissure of the beak and continues to the ventral side of the neck, lateral to trachea, up to the level of the cloacal orifice. With scissors, we cut transversely the abdominal muscles from the posterior part of the sternum, posterior to the xiphoid appendix. On each side of the sternum, the initial abdominal incision continues, up to the level of the chondrocostal junctions. The abdominal wall is sectioned longitudinally, up to the cloaca and broken laterally. We cut the chondrocostal junctions with scissors, bilaterally, up to the level of the scapulohumeral joints; the coracoid bones and the clavicle are cut, the sternum is removed, after the pericardial sac is disintegrated. The organs located in the cavity are detached, both commissures of the beak were cut, with the lower jaw detached, together with the oesophagus and a portion of the trachea. The skin and the musculature adjacent to the cloacal orifice were cut in order to detach it, together with the digestive tract and the attached organs.

The digestive tract was examined only macroscopically, *in situ*, and separately from the carcass. We cut and opened the oesophagus, the glandular stomach, the shredding stomach, the small intestine, the large intestine, the cloacal orifice.

The first measurements were made before the body was opened, using the electronic scale and a measuring tape. The bodies were measured from the top of the head to the top of the tail. After opening the bodies, the entire digestive tract was isolated and weighed. The liver was isolated from the digestive tract, weighed and measured. The pancreas was identified in few bodies, so it was excluded from the measurements. The entire digestive tract was weighed, without liver, following the measurement of the digestive tract in segments: oesophagus, glandular stomach and gizzard together, then small intestine and large intestine, also together.

A digital camera, Nikon COOLPIX P900, was used to collect the images.

Results and discussion

Data regarding measurements of the digestive system of the two species can be found in Table 1.

Table 1. Digestive tube measurements (BW - Body weight, BL - Body lenght, DtWL - Digestive tube weight with liver, L- Liver, we – weight, le – lenght, wi – width, DtW - Digestive tube weight without liver, AD - Anterior digestive tract, PD - Posterior digestive tract (*anterior digestive tract – esophagus, proventriculus (glandular stomach) and the gizzard (muscular stomach); **posterior digestive tract – small intestine and large intestine)

		Ciconia ciconia				*Buteo buteo*			
		Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
BW(g)		2421	1885	2900	371.8	829.2	600.2	1012.8	153.8
BL(cm)		77.2	73	83	3.76	48.6	47	51	1.62
DtWL(g)		350.8	313	400	39.63	39.86	43.8	56	4.86
L	we (g)	50.4	40	59	6.28	14	8	18	3.63
	le(cm)	3.4	5	6	0.37	5.6	5	6	0.37
	wi (cm)	6.6	6	7	0.48	5.4	4	7	1.01
DtW(g)		300.4	273	352	40.35	34.16	32.5	40	4.69
AD* (cm)		49	45	61	6.09	15.6	15	25	3.46
PD** (cm)		147.8	115	171	24.86	129	120	135	4.93

Beak, tongue and oral cavity

The beak of Common Buzzard is strong and curved; it includes parts of the upper maxilla and lower maxilla. It was observed that the upper maxilla is better developed compared to the lower maxilla. This anatomical feature was described by Lacasse (2015), Denbow (2000), Murray (2014) and Ford (2010). On the surface, the beak is covered by several layers of hard keratin, scientifically called rhamphotheca, a peculiarity of the Aves class. Part of the rhamphotheca which covers the maxilla is called the rhinotheca, and the segment which covers the mandible is the gnatotheca (Denbow, 2015). At the base of the jaw of rhamphotheca, we identified a fleshy formation called the ceroma. This is the boundary between the beak and the front part of the head and includes the two nostrils. The dorsal median limit of the maxillary rhamphotheca is called the culmen, and the ventral median limit of the mandibular rhamphotheca is called gonys. The sharp edge of the rhamphotheca is called tomia. The anatomical name of these regions in the beak structure were mentioned by Speer and Powers (2016), Denbow (2015) and McLelland (1990). Also, we noticed that the soft palate and oropharyngeal isthmus is absent in common buzzard. Due to this anatomical peculiarity, a common cavity is created, the oropharynx (common characteristic of the Aves class) (Fig. 1). At the level of the hard palate, we identified two longitudinal cracks that connects the nasal cavity to oropharyngeal cavity - the choana. At short distance, aboral from the choana, in the bottom part of the pharyngeal ceiling, we identified the infundibular cleft, which is medially located and is the common opening to the auditory tubes (Fig. 2). All these features are mentioned by Denbow (2015), Speer and Powers (2016), McLelland (1990), Klassing (1999).

White storks have a long and straight beak with the tip of the beak slightly sharp. The upper maxilla and the lower maxilla have the same length, the rhamphotheca is well formed. The beak of the white storks has no sharp edge, as can be seen in the common buzzard, nor the tomial tooth. At the base of the upper rhamphotheca ceroma was not identified, the nostrils are elongated and included in the structure of the upper maxillary, bounded by the rhamphotheca (Fig. 3). The soft palate and oropharyngeal isthmus is absent. The choana was identified at the level of the hard palate, presenting on its surface the openings of the salivary glands; posterior from choana we identified the infundibular cleft. These features were described by McLelland (1990), Fidget and Dierenfeld (2008).

Figure 1. The beak of *Buteo buteo* (a. Rhinotheca: 1.The tip of the curved beak 2.Culmen, 3.Ceroma and nostrils; b. Gnatotheca: 4.Gonys, 5.Tomia)

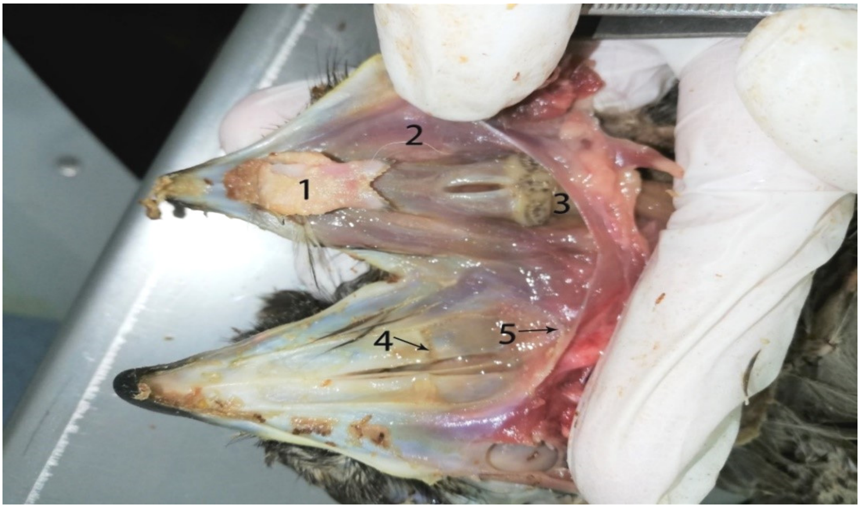

Figure 2. Oropharynx (Jaw: 1. Tongue; 2. Laryngeal protuberance with glottal orifice; 3. The esophageal orifice; Maxilla: 4. Choana; 5. Infundibular fissure, The presence of cornified papillae is indicated with the help of arrows)

Figure 3. The appearance of the beak of White stork (*Ciconia ciconia*) (a: Rhinotheca. 1- The tip of the beak slightly sharp, no tomial tooth 2- Elongated nostrils, bounded by rhamphotheca, b: Gnatotheca, free of gonys)

Oropharyngeal cavity

Common buzzard has in the oropharyngeal cavity, on the surface of the palatine ridges, tongue and glottal process, cornified epithelial projections; on tongue, these cornified papillae are arranged in the form of the letter V, with an aboral opening (Fig. 4). These characteristics were also reported by Ford (2010),

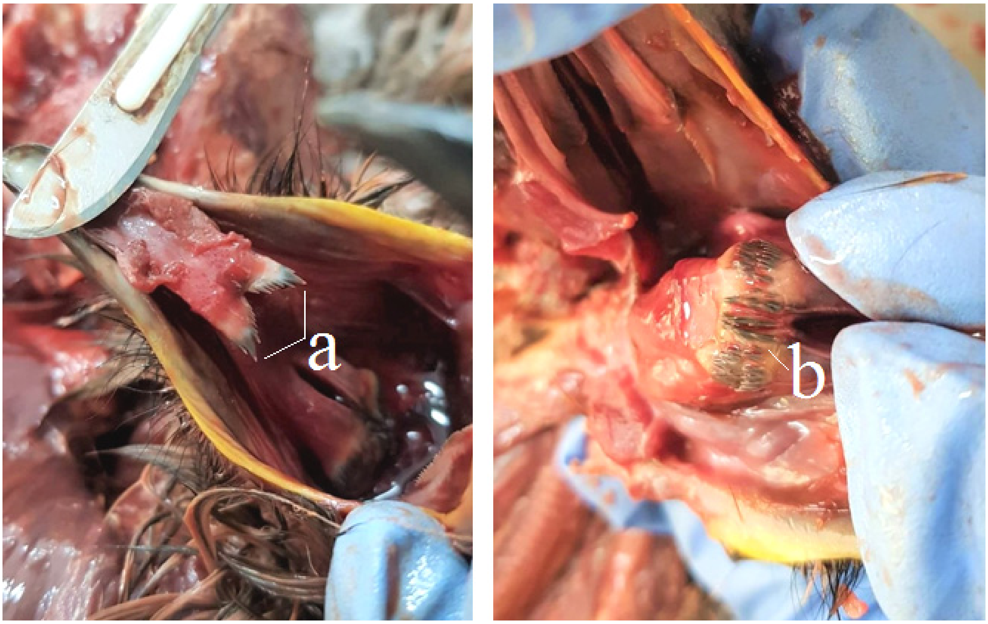

Figure 4. The cornified papillae in common buzzard (*Buteo buteo*) (Present on the surface of the tongue, oriented in V (a) and cornified papillae located on the surface of laryngeal protuberance (b))

claiming that raptors have a greater limb mobility compared to parrots, whose tongue is thicker and poorly mobile. White storks have a short, immobile tongue, resembling with a rounded spear tip, without cornified formations on the surface, which continues with a well-developed laryngeal protuberance (Fig. 5). At the level of the lower maxilla, close to the half of the oropharyngeal cavity and the tip of the tongue, longitudinal folds are present and can increase the volume of the cavity. These peculiarities were also described by Fidget and Dierenfeld (2008) (Fig. 5).

Larynx

In the inspected birds, the larynx has a laryngeal protrusion with an oro-aboral opening slit, representing the tracheal entrance (Fig. 6). In the common buzzard, the esophagus is short and has two part: cervical part, which is reduced, and the thoracic part, more developed (Fig. 7). This particularity is also mentioned in the literature by Ford (2010) and Denbow (2000). In the last segment of the oesophagus, we identified a crop, an expansion of the oesophagus, identified in all common buzzards we have studied (Fig. 8). The crop is continued

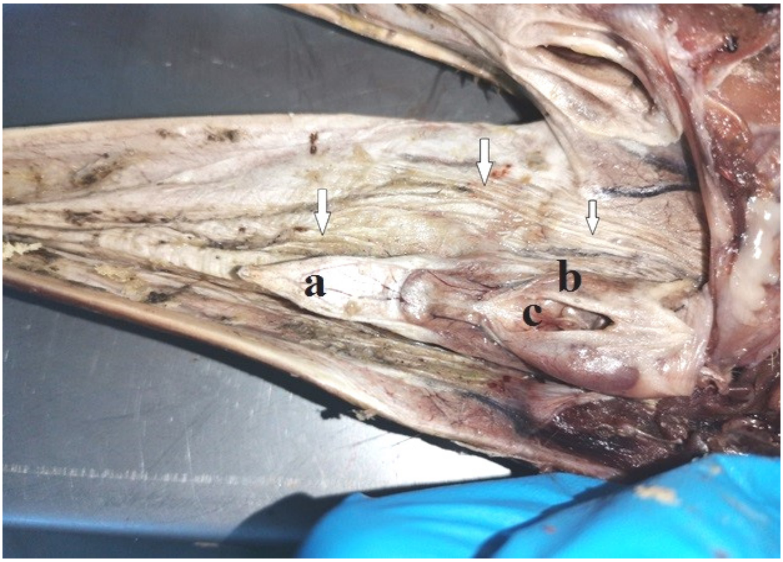

Figure 5. The appearance of lower maxilla in white stork *(Ciconia ciconia*): the presence of longitudinal folds at the level of the lower maxilla indicated with arrows and the short and immobile tongue (a), continued by the laryngeal protuberance (b) and the entrance in the trachea (c)

Figure 6. Appearance of the oropharynx cavity with laryngeal protrusion (a) and entrance in the trachea (b) in common buzzard (first image) and white stork (second image)

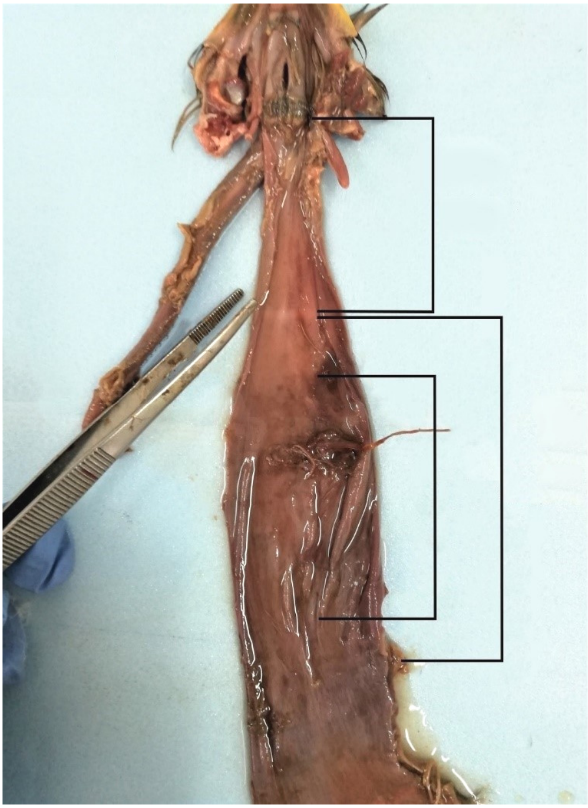

Figure 7. (left) Delimitation of the esophagus in common buzzard, highlighting the crop, which is located in the last esophageal segment
Figure 8. (right) Highlighting the length of the esophagus in white stork

with the glandular stomach through a demarcation represented by a poorly developed sphincter. In the literature, Houston and Duke (2005) recall the presence of a poorly developed crop in raptors and the absence of this anatomical segment in owls. In white storks, the oesophagus has logitudinal folds among its entire length - it is long and distensible, without a crop (Fig. 9 and Fig. 10). Near the glandular stomach the folds fade and become a girdle (Fig. 10). These features were also highlighted by McLelland (1990) and Fidget & Dierenfeld (2008).

Figure 9. The absence of the crop and the appearance of longitudinal folds of the esophagus in white stork.

Stomach

Common buzzards have a small glandular stomach, interconnected with the thin wall of the ventricle. Both structures -glandular and muscular stomach- form a pear-like shape structure. This aspect is also mentioned by Ford (2010), which makes a comparison between the degree of development of the proventriculum and ventriculum in raptors, compared to Psittaciformes or other granivores. The proventriculus and gizzard are separated by a girdle, which is poorly represented in raptors (Fig. 10). Ford (2010) claims that this isthmus is absent, the passage between the ventricles is represented by a cavity. Denbow (2000) reports the presence of a girdle between the glandular and muscular stomach, both cavities are developed differently, depending on the species. The pyloric region connects the gizzard with the duodenum and it

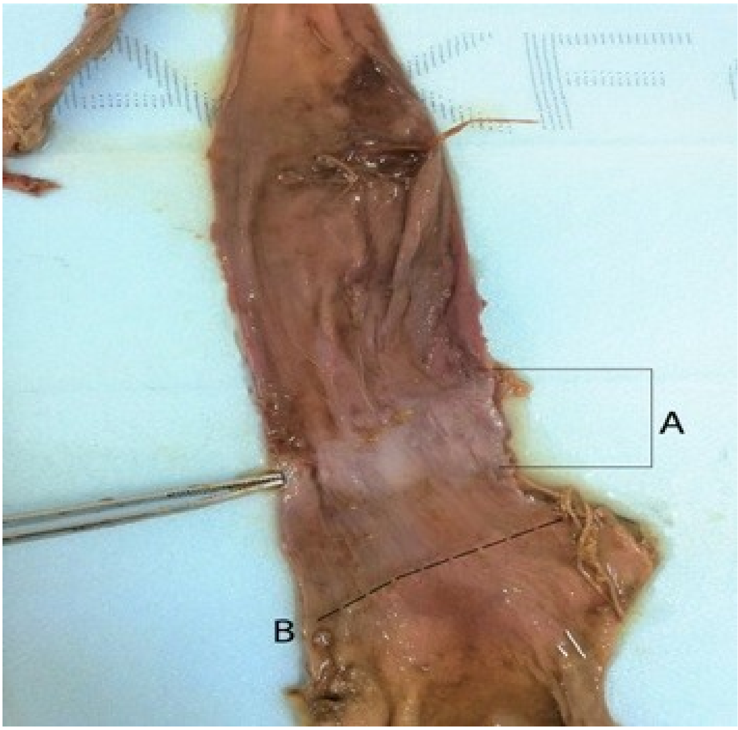

Figure 10. The passage between the glandular stomach (A) and the muscular stomach (B) is dominated by an isthmus or girdle (indicated with continuous line)

is located on the right side of the organ, 90° from the longitudinal axis of the muscular stomach. White storks have a poorly developed glandular stomach and on the surface of the mucosa we observe numerous channels of the secretory glands (Fig. 11). The passage between the glandular and muscular stomach is represented by a girdle or isthmus (Fig. 11). From this level, the mucosa of the muscular stomach is folded and covered by koilin. In the inspected bodies, koilin has a brownish-green color (Fig. 11). The pyloric region is located close to the isthmus between the two gastric parts, at about 45° from the axis of the oesophagus-glandular stomach-gizzard. Unfortunately, we did not find information in the literature to make a comparison.

Figure 11. The appearance of the two compartments of the stomach in white storks: the fading area of the esophageal folds near the proventriculus (a), the glandular stomach or proventriculus (b), the isthmus marking the area of passage from the origin to the ventricle (c) and the folded appearance of the mucous membrane of the muscular stomach, covered with koilin

Intestines

In common buzzard, the intestinal mass is compact, positioned in the caudal portion of the thoraco-abdominal cavity, surrounded by adipose tissue (Figs. 12 and 13). The small intestine is short and it extends from the level of the pyloric region of the ventricle to the level of the cecum and colon (Fig. 14). This is also mentioned by Ford (2010), Murray (2014), Houston and Duke (2005). Ford (2010) states that the duodenum - the first segment of the small intestine - is long, aspects also identified in the studied birds, and some species (birds of the genus Haliaeetus, family Accipitridae) have secondary loops. The duodenal portion is long, without an obvious delineation between duodenum and ileum. Murray (2014) also supports the presence of additional loops and performs the following classification:

- duodenal loop, contains the pancreas (Ford, 2010); it is elongated in hawk and eagles (King, McLelland, 1984, quoted by Murray, 2014)
- the axial loop presents Meckel's diverticulum; at this level, the transition between jejunum and ileum occurs (King, McLelland, 1984, quoted by Murray, 2014)
- the supraduodenal loop - the most distal loop of the ileum, is located dorsally from the duodenum (King, McLelland, 1984, cited by Murray, 2014)
- the supracecal loop, presented only in a few species of birds, is located near the ileo-rectal junction (King, McLelland, 1984, quoted by Murray, 2014).

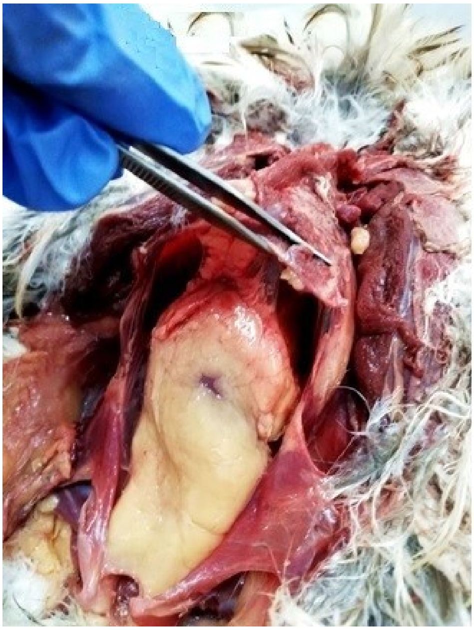

Figure 12. (left) Adipose tissue that incorporates the digestive tract of common buzzard

Figure 13. (right) Topography of organs in the thoraco-abdominal cavity in common buzzard after removing the adipose tissue: 1. Heart and pericardial sac 2. Hepatic lobes 3. Small intestine, compact mass 4. Ventricle or muscular stomach 5. Colon

Figure 14. Approximative delimitation of the digestive tract in common buzzard (A. Esophagus with the crop, glandular stomach and muscular stomach; B. Small intestine, without a clear demarcation of the duodenum, jejunum and ileum. C. Large intestine, with colon, cecum and cloaca)

In white storks, the intestinal mass is compact, positioned in the caudal portion of the thoraco-abdominal cavity (Fig. 15). The small intestine is long, extending from the level of the pyloric region of the ventricle to the level of the ileo-ceco-colic junction (Fig. 16). The small intestine is folded into numerous loops, with the help of mesentery – there is no clear delimitation between segments of the small intestine (Fig. 17).

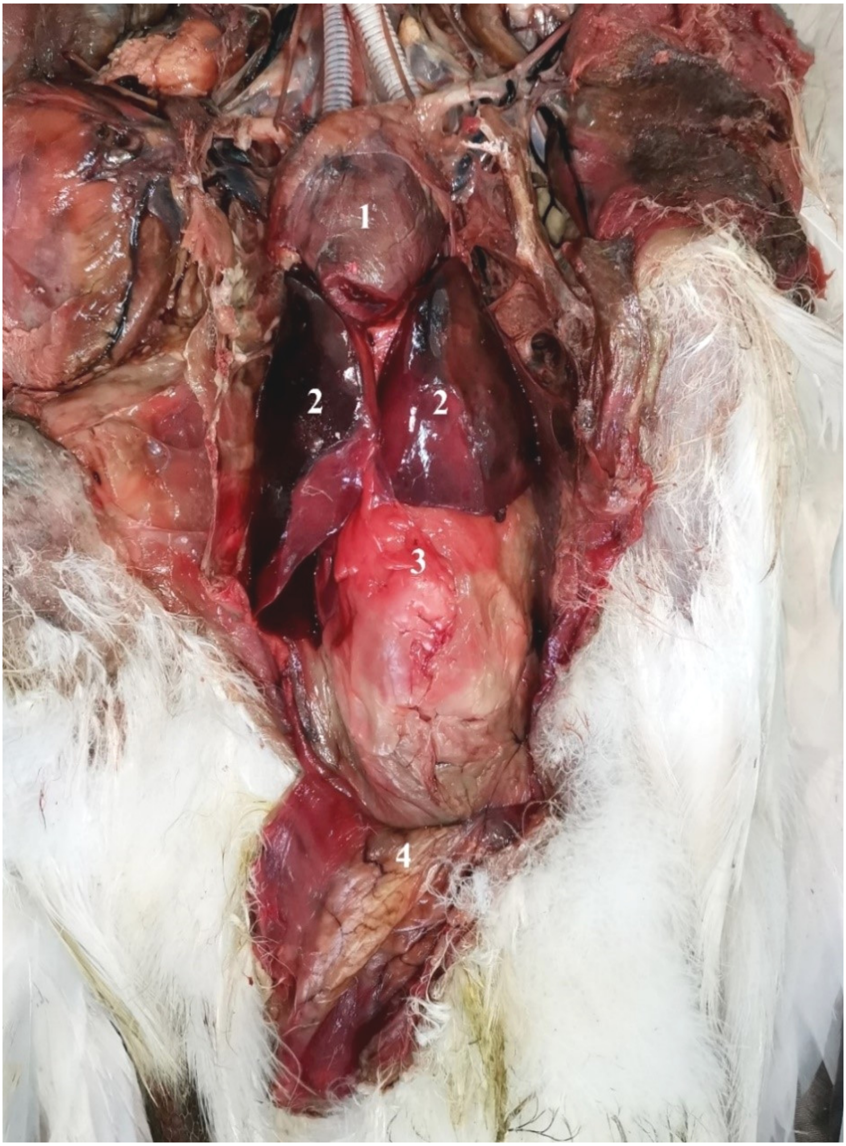

Figure 15. Topography of organs in the thoraco-abdominal cavity after removal of adipose tissue in white stork: 1. Heart and pericardial sac 2. Hepatic lobes 3. Muscular stomach 4. Colon

Figure 16. Intestine of *Ciconia ciconia*: delimitation of the small intestine from the level of the pyloric region of the ventricle (a) to the ileo-ceco-colic junction (marked by arrows), highlighting the location of the pancreas in the first part of the duodenum, near the pyloric orifice (b), the delimitation of the colon (c) continued by the cloaca (d) that opens outside through the anal orifice.

Cecum and cloaca

In the common buzzard, the ceca is represented by two rudimentary vestigial formations, observed in all the studied birds, aspects mentioned by Murray (2014), Clench (1999) and McLelland (1989), identified as a lymphoid-type ceca (Fig. 18). The colon is short, extending from the level of ileo-cecale junction to cloaca, as reported in the literature by Murray (2014) and Ford (2010). The cloaca extends from the distal portion of the colon to the anal orifice (Fig. 19). In white storks, the ceca is small in size, vestigiale, lymphoid, characteristic for the birds of the order Ciconiiformes. This was pointed out by Clench (1999) and McLelland (1989). The colon is short and opens outwardly through the anal orifice (Fig. 19).

Figure17. Part of small intestine folded with the help of mesentery in a white stork; blood vessels can be seen in the structure of adipose tissue and ligament

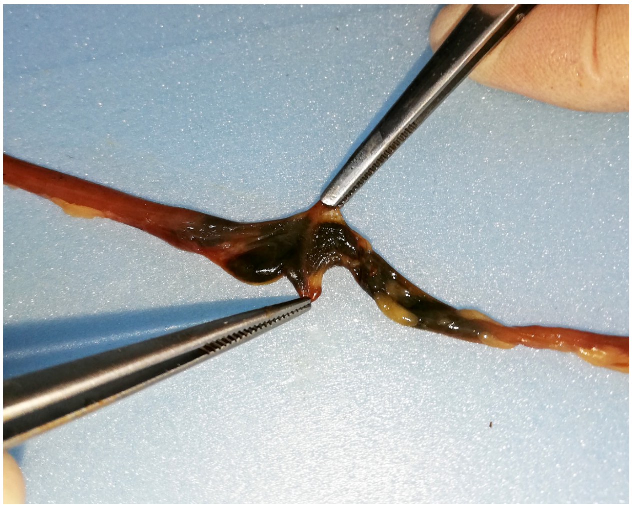

Figure18. Cecum in a common buzzard: represented by the two rudimentary formations, characteristic for the birds of the order Accipitriformes

Figure 19. Delimitation of the large intestine in common buzzard: at the junction between the ileum and the colon (A)we identified the ceca (arrow).The colon continues with the cloaca and the cloacal orifice (B).

Pancreas

We didn't identify the pancreas in common buzzard bodies due to the quality of the preserved corpses. In white stork corpses, we identified the elongated-looking pancreas, located in the first part of the duodenum, near the pyloric orifice (Fig. 20).

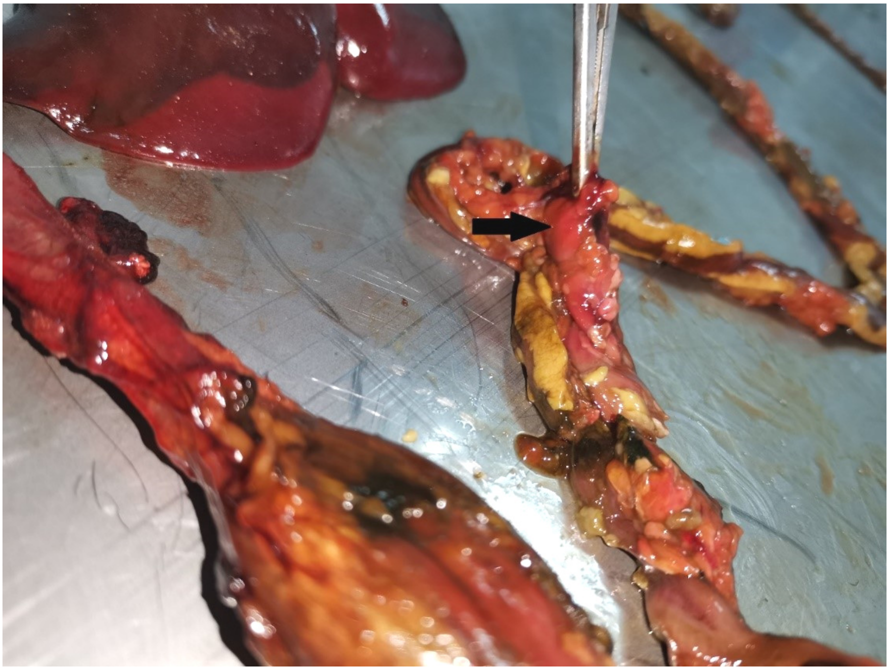

Figure 20. The presence of the pancreas in a white stork, indicated with an arrow and its localization in the first part of the duodenum.

Liver

In common buzzard, the liver have two lobes joined cranially in the midline, located in the thoracic-adominal cavity; the lobes surround the apex of the heart (Fig. 21). Similar data describing the location of this organ was reported in the literature by Samour and Naldo (2007). The right lobe is larger compared to the left lobe (fig .22), aspects also found in the descriptions of Samour and Naldo (2007), while Murray (2014) does not consider this to be a peculiarity for the raptors. All the studied bodies have a gallbladder, located on the ventral side of the right lobe, well developed (Fig. 22). The presence of the gallbladder and the topography of the liver is confirmed by Murray (2014), Samour and Naldo (2007), Klaphake and Clancy (2005).

In white stork, the liver has also two lobes that join cranially on the midline, located in the ventro-cranial part of the thoraco-abdominal cavity and surrounds the apex of the heart (Fig. 22).

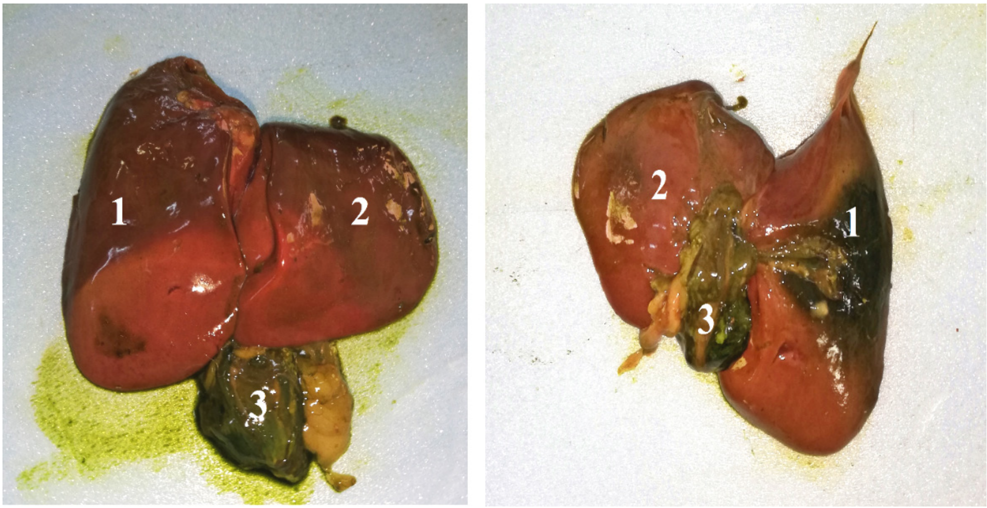

Figure 21. The liver in common buzzard (*Buteo buteo*): the two hepatic lobes, the right lobe (1) visibly larger than the left one (2) and the gallbladder (3), the view from the parietal part (the first picture) and the visceral face of the liver (second picture)

Like the common buzzard, the liver of storks has two lobes of different sizes, the right lobe being more developed compared to the left lobe (Fig. 23). All corpses of white stork have a poorly developed gallbladder, located on the visceral side of the right lobe, covered with fat tissue (Fig. 22).

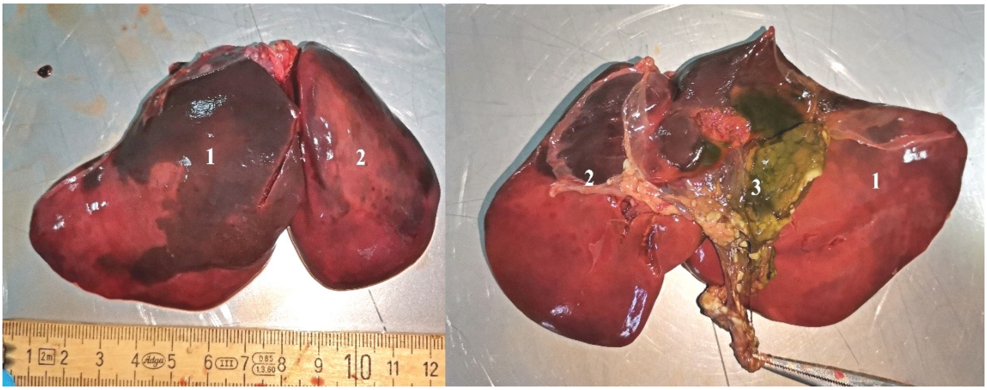

Figure 22. The two hepatic lobes in white stork (*Ciconia Ciconia*): the right lobe (1) visibly larger than the left one (2) and the gallbladder (3), the view from the parietal part (the first picture) and the visceral face of the liver (second picture)

Conclusions

We concluded that the digestive system is adapted to a strictly carnivorous diet. The differences identified between the anatomical structures of the digestive tract of the two species are correlated with the differences that appear in their feeding behavior.

The beak is showing visible differences, in common buzzard the beak is strong, curved, adapted for the laceration of prey. In white stork the beak is long, strong, straight, the upper maxilla is about the same length as the lower maxilla, adapted to capture prey by hitting it like a harpoon.

In common buzzard the tongue is slightly mobile, and on its surface we identified the cornified papillae. The absence of the soft palate created a common cavity with the pharynx (oropharyngeal cavity). In white stork the tongue is immobile, located on the floor of the oro-pharyngeal cavity, without cornified papillae on its surface, looking like a spearhead.

In both species the esophagus is located on the right side of the neck, it can widen its diameter due to longitudinal folds. Obviously, the length is different, directly proportional with the neck, longer in white storks, shorter in common buzzards. In common buzzard, the terminal part of the oesophagus shows a dilation, the crop, located cranially in the chest cavity, which has the role of storing the ingested food. The crop is absent in white storks.

In both species, the proventriculus and ventriculus are poorly developed in comparison with non-carnivorous birds. The koilin layer is better highlighted in white stork compared to common buzzard.

In common buzzard, the small intestine is reduced in size, without obvious macroscopic transition between the duodenum and the ileum. The white storks have a long small intestine with many loops, without a transition between the segments (duodenum, jejunum and ileum).

In both species, the ceca is reduced in size and has a vestigial appearance; the colon is reduced in size and it extends from the level of the ileo-colic junction to cloaca.

The liver has two lobes, the right lobe is well developed in comparison with the left lobe. In common buzzard, the gallbladder is well developed, located on the ventral side of the right lobe; in white storks, the gallblader is poorly developed, located on the ventral side of liver, the visceral face of the right hepatic lobe.

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Preliminary data regarding the influence of the COL1a1 *rs2249492* polymorphism on the risk of malocclusion in the Romanian population

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Abstract. Malocclusion is a condition characterized by diverse phenotypic expression patterns, with a complex underlying genetic background. COL1a1 is one of the genes that has been previously associated with malocclusion, with one particular SNP, *rs2249492* (C>G, C>T), having been linked with an increased risk of skeletal class II malocclusion.

In this paper, making use of DNA sequencing and cephalometric measurements, we present preliminary data regarding the association between the *rs2249492* SNP and the risk of malocclusion in the Romanian population, illustrated as continuous, rather than categorical phenotypes. The results show a tendency towards a Class II pattern determined by mandibular retrognathism, rather than maxillary prognathism among the individuals possessing the mutant allele. Subsequent studies on larger sample sizes should include statistical analysis focused on associations between the *rs2249492* allele and continuous phenotypic variation inside, but not restricted to Class II malocclusion, in order to acquire a more detailed picture of the interaction between the polymorphism and this complex condition.

Keywords: malocclusion, COL1a1, *rs2249492*, cephalometric measurements, SNP

Introduction

The term malocclusion encompasses various phenotypic expression patterns, caused by a complex genetic background and characterized by the unharmonious growth of the maxillary and mandibular structures, leading to a defective relationship between the dental arches (Laviana *et al*., 2021; Nishio and Huynh, 2016; Weaver *et al.,* 2017). According to Angle's classification, this complex condition can be divided into three groups – Class I, II and III. The last two types are the most frequent, impacting not only the patient's masticatory functions, but also their appearance and mental health, leading to a reduced quality of life in affected individuals (Graber *et al*., 2017; Li *et al*., 2010; Liu *et al*., 2009; Ma *et al.,* 2019).

Class II malocclusion encompasses phenotypes characterized by a convex facial pattern, as a consequence of either a protruded maxilla or a retruded mandible. Conversely, Class III phenotypes are characterized by concave facial profiles caused by either a retruded maxilla or a more protruded mandible, of which the most well-known is mandibular prognathism (Doraczynska-Kowalik *et al*., 2017; Hardy *et al*., 2012; Laviana *et al*., 2021; Li *et al*., 2010; Liu *et al.,* 2009).

A good, detailed understanding of the genetic factors underlying malocclusion is vital so that orthodontists are able to correctly diagnose and treat this condition (Weaver *et al.,* 2017; Zabrina *et al.,* 2021). This study is concerned with the COL1a1 gene, that encodes the pro-alpha chain of type I collagen and has been previously associated with malocclusion (Da Fontoura *et al*., 2015; Doraczynska-Kowalik *et al.,* 2017; Zabrina *et al.,* 2021). Mutations of this gene have been linked with various diseases, such as those related to facial anomalies and osteogenesis imperfecta. Out of these mutations, one particular SNP, *rs2249492* (C>G, C>T), has been linked with an increased risk of skeletal class II malocclusion (Da Fontoura *et al.,* 2015).

As such, this paper is aimed at presenting preliminary data regarding the influence of the *rs2249492* polymorphism on malocclusion risk in the Romanian population, with focus on continuous phenotypic variation represented by four different cephalometric measurements.

Materials and methods

2.1 Cephalometric measurements

In order to obtain preliminary data on the association between the *rs2249492* allele and malocclusion in the Romanian population, the sample set comprises a total of 12 individuals, of which 9 suffer from Class II malocclusion and 3 suffer from Class III, classified according to the clinical evaluation, facial photographs evaluation, dental cast study and cephalometric measurements.

With the purpose of getting a more detailed picture on the patients' complex phenotype, four cephalometric measurements performed in the OnyxCeph software and assessed with Steiner analysis and Witts appraisal were taken into account: the SNA, SNB, ANB and AoBo (the Witts appraisal). The SNA and SNB angles are measures of maxillary and mandibular position, respectively, while the ANB and AoBo are measures of jaw disparity (Ghergie *et al.,* 2013).

2.2 DNA extraction, PCR amplification and DNA sequencing

Genetic data were collected from the same sample set mentioned above.

Genomic DNA was extracted from buccal swabs, using the Animal and Fungi DNA Extraction Kit (Jena BioScience, Germany), according to the manufacturer's instructions.

PCR amplification and Sanger sequencing (Macrogen Europe, The Netherlands) were carried out on a fragment of the COL1a1 gene containing the *rs2249492* SNP. The primers amplified a region of 500 bp length and had the following sequence (Da Fontoura *et al.*, 2015; Doraczynska-Kowalik *et al.,* 2017):

COL1a1_F	GTAAGGTTGAATGCACTTTTGGTTT
COL1a1_R	GTGAGTGCCCAGAATCCCCA

The PCR reaction setup that led to the obtained results was as follows: 25µL total reaction volume, 1×PCR Buffer, 2.5 mM MgCl2, 0.2 mM dNTPs (Bioline, Meridian Bioscience, USA), 0.5 pM of forward and reverse Primers and 1.25 units/reaction MangoTaq Polymerase (Bioline, Meridian Bioscience, USA). The PCR parameters were: 95°C for 5 min (initial denaturation), followed by 35 cycles of 95°C for 30 s (denaturing), 66°C for 30 s (annealing), 72°C for 30 s (extension) and a final extension for 5 min at 72°C.

Results and discussion

The obtained results indicate a high tendency towards the mutant T allele in the investigated cohort, with 7 out of 9 Class II patients and all 3 Class III patients having this SNP, and only 2 Class II individuals having the reference C allele. Complete cephalometric measurements were obtained for 9 out of 12 individuals and the results are presented below, in Table 1.

When looking at the Class II group, the results are indicative of the previously confirmed association between the *rs2249492* T allele and an increased risk of developing Class II malocclusion (Da Fontoura et al., 2015). What can also be noted is the fact that all 3 members of the Class III group have the same mutant T allele. However, further inquires on larger sample sizes are necessary in order to confirm an association between the *rs2249492* SNP and Class III malocclusion.

Next, when analyzing the results of the cephalometric measurements, complex phenotypes can be observed, suggesting that the individuals' Class II and III skeletal pattern may not be simply described as either mandibular or maxillary prognathism.

The SNA angle, which indicates the position of the maxilla, has a mean value of 82º, with higher values indicating maxillary prognathism and lower values representing maxillary retrognathism (Doraczynska-Kowalik *et al*., 2017; Ghergie *et al.,* 2013). In the case of the Class II group, while 2 patients display SNA values lower than the mean, 3 have values around the mean and 1 has a value higher than the mean. For the Class III individuals, two highly different measurements were obtained, with one having an above-the-mean value and the other, lower than the mean.

The SNB angle, which indicates the position of the mandible, has a mean value of 80º, with mandibular prognathism being reflected by higher values and mandibular retrognathism by lower values (Doraczynska-Kowalik *et al*., 2017; Ghergie *et al*., 2013). In this case, again, the Class III individuals have opposing values, while the Class II group is characterized by values indicative of mandibular retrognathism.

Lastly, the ANB (mean of 0-2º) and AoBo (mean of 0-2 mm) angles, that are used to assess the level of jaw disparity (Doraczynska-Kowalik *et al.,* 2017; Ghergie *et al.,* 2013), reinforce the idea of complex malocclusion phenotypes. While all Class II individuals can be classified as such on the basis of the ANB, only 4 present a Class II skeletal pattern on the basis of the Witts' appraisal. As far as the Class III individuals are concerned, the ANB values suggest opposing skeletal patterns, while the AoBo values are indicative of Class III skeletal patterns.

Considering how essential it is to understand the complex genetic background of this condition in order to establish better preventive measures as well as efficient, personalized therapies (Dehesa-Santos *et al.,* 2021; Moreno Uribe and Miller, 2016; Xue *et al.,* 2010), attention needs to be paid to the correlations between genotype and continuous phenotypic variation (Da Fontoura *et al*., 2015). Of course, given the small sample size of this preliminary study, no statistically significant associations could be found, however a tendency towards a Class II pattern driven by mandibular retrognathism, rather than maxillary prognathism among the individuals possessing the mutant allele can be observed. Further studies on larger sample sizes should include statistical analysis focused on associations between the *rs2249492* allele and continuous phenotypic variation inside, but not limited to, Class II malocclusion, in order to obtain a more detailed picture on the interaction between the SNP and this complex condition (Da Fontoura *et al*., 2015).

Table 1. *rs2249492* alleles and cephalometric measurements.
Grey areas signify a lack of data.

Nr. crt.	**Class**	**Allele**	**SNA º**	**SNB º**	**ANB º**	**AoBo (mm)**
1	II	C	78.3	70.2	8.1	3
2	II	T	81.9	74.3	7.7	3
3	II	T	82.2	76.9	5.4	-2
4	II	C				
5	II	T				
6	II	T	80	74	6	1.96
7	II	T	83.3	77.5	5.8	3
8	II	T	82.1	77.8	4.3	3
9	II	T	79.7	76.2	3.5	1
10	III	T	83.4	81.7	1.7	-6
11	III	T				
12	III	T	74.4	70.4	4	-3

Conclusions

This paper offers promising preliminary data regarding the influence of the COL1a1 gene on the risk of developing malocclusion in the Romanian population, as well as a useful experimental setup to be used in further studies of this kind.

While there have been previous discussions regarding the different prevalence of malocclusion across distinct geographic areas (Da Fontoura *et al.,* 2015; Hardy *et al*., 2012), studies on the region of Romania have yet to be included (Ghergie *et al.,* 2013). As such, our study presents initial data that can be utilized as starting hypotheses for more extensive studies focused on the population of Romania.

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Ethic statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca (97/08.03.2017). Informed consent was obtained from all subjects involved in the study.

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Aspects from the evolution of past vegetation in Southern Transylvania (Sibiu region)

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Abstract. The article reviews the results obtained through the palynological study of sequences obtained from the Sibiu region and adjacent areas. The oldest Upper Pleistocene sequences we refer to seem to reflect snapshots from the Last Glacial Maximum. The history of the Late Glacial and Holocene vegetation here is broadly part of the succession known for the territory of Romania, in the Carpathians and the surrounding areas of medium and low altitudes. The dynamics of the main forest and grasses taxa were highlighted, influenced both by natural factors and, more recently, by human activity. The reference area is characterized by the domination of the forests during the interstadials and by open landscape of forested steppe during the cold, stadial periods.

Keywords: Palynological method, vegetation dynamics, influencing factors

Introduction

Paleo-vegetation studies using the palynological method are a useful tool in deciphering the evolution of vegetation over time. The most suitable environments for the conservation of pollen and fossil spores are bogs and swamps, but also lake environments that have met the storage conditions.

Palynology studies in Romania, initiated at the beginning of the last century, have gone through several stages of development. At first no emphasis was placed on the identification of grass taxa in the pollen spectra. In addition, these studies have suffered, for decades, from the lack of an absolute chronology provided by ^{14}C dating. Lately, more and more emphasis is being placed on the detailed identification of anthropogenic indicators, charcoals (micro and macro), and on the correlation of these studies with other types of research, to ensure an interdisciplinary character and to increase the level of knowledge. In his pioneering works, Pop (1928, 1929, 1932, 1942) sketched the general scheme of the evolution of the vegetation on the Romanian territory. Thus, in 1928 the first rigorously scientific pollen diagram elaborated in our country is presented. The palynological analysis of the old peat from Colăcel (800 m altitude) showed that during its deposition, the surrounding forest was composed of pine (*Pinus* sp.), with disseminations of spruce (*Picea abies* Karst.), willow (*Salix* sp.), and birch (*Betula* sp.). This "*phase of the pine forest*" was, for the first time, highlighted during the Holocene in our country. Pop also finds in this sediment the so-called "*grenzhorizont*", the level of drying and afforestation of swamps, highlighted throughout Europe. Pop makes a comparison with northern Europe, where three stages are distinguished in the warm Postglacial period: the warm and dry Boreal, the warm and humid Atlantic, and the warm and dry Subboreal. A cold and humid period follows, the Subatlantic, which we are still going through today (periodization cf. Blytt, 1876, 1882, and Sernander, 1890). These climatic oscillations correspond to a dynamic of vegetation that Pop has been sketching ever since. Thus, in 1929, the author describes in his doctoral thesis the following succession of forest and climates: a). *Pine phase* in Preboreal; b). *Pine-spruce phase with traces of hazel and mixed oaks* in the early Boreal; c). Boreal-Atlantic *mixed spruce-hazel-oak phase*; d). *Spruce-hornbeam phase* in the Subboreal; e). *Spruce-beech-fir phase* in the Subatlantic. The history of the Holocene vegetation in the Sibiu region (Fig. 1) is broadly part of this succession described for the Romanian territory.

Results and Discussion

General framework

The relative chronological framework established by Pop (1928, 1929) for the evolution of the postglacial vegetation on the Romanian territory constitutes a starting point for the similar approaches that succeeded it. In addition to establishing some forest phases, Pop (1929) also stated some important conclusions, valid for the evolution of vegetation in Romania, which have demonstrated their viability. Thus, the author underlined the great influence that glaciation had on the vegetation, determining the lowering of the tree line towards the plain and the uniformization of the forest aspect; the genus *Pinus* was present on very large areas; the belts of forest vegetation, as they are known today, did not exist then. The end of the glaciation and the warm weather that followed changed the composition of the forests. Pine retreated to the mountain belt, spruce became dominant, mixed oaks and hazel vegetated at altitudes with higher amplitude. An altitudinal layering appeared (mixed pine-spruce-oak), in which hornbeam was also integrated, forming in the Subboreal period a narrow and interrupted area, between spruce and oak. The beech belt is the youngest and was formed in the Subatlantic period, completing the forest landscape. The evolution of forests is qualitatively similar at different altitudes, in the sense that the same genera appear and expand in approximately the same order, while the differences are mostly quantitative.

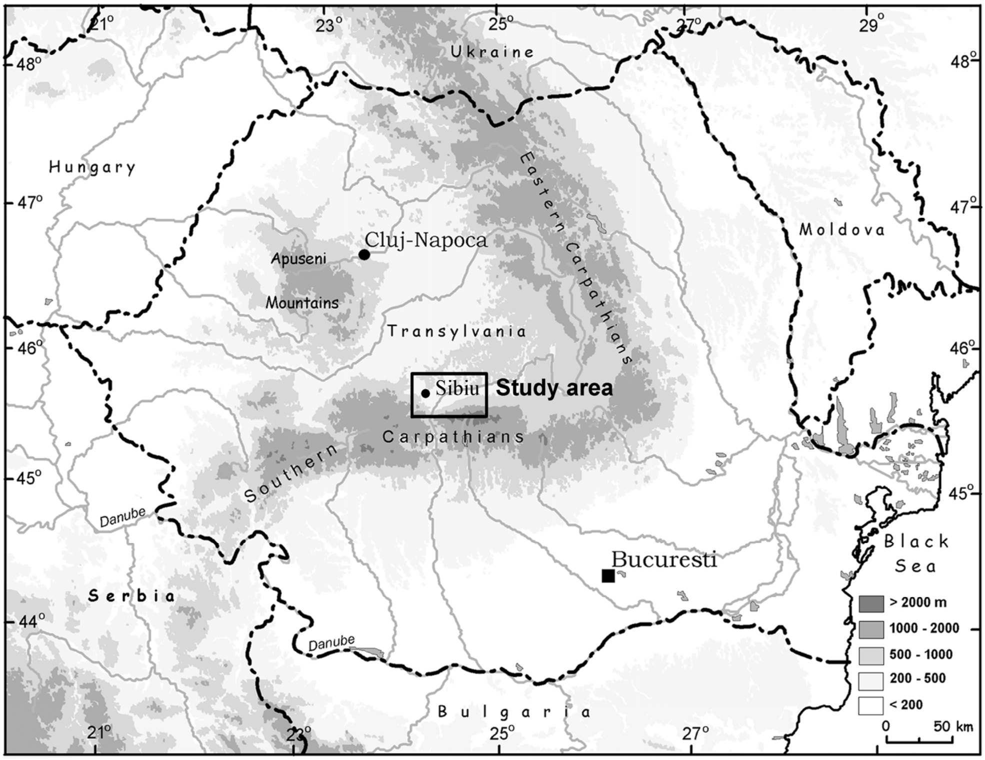

Figure 1. Location of the study region

Pop (1942) goes even further with detailing the evolution of vegetation, establishing three sub-phases for the phase of *spruce with hazel and mixed oaks: the first peak of mixed oaks (especially elm) and spruce; the absolute maximum of mixed* *hazel and oak* (at the peak of the warm postglacial weather); *the absolute maximum of the spruce*. The author anchors our forest evolution in the general central European framework, underlining the differences. In the Romanian Carpathians and the adjacent lower areas, arid/dry pine forests were preserved during the last glaciation, also housing spruce. Other reported features are the delay of hazel, fir and especially the insertion of the hornbeam phase between the spruce phase and the beech phase. Pop states that most of our thermophilic tree elements are relics of the warm Postglacial period and not of the Tertiary period. Studies conducted by Pop at the time confirm the view that glaciations, interglacials, and the Postglacial period led to profound transformations, migrations, removal, and colonization of species. In addition, based on results obtained until that date, the author draws the conclusion that "*the main engine of forest variations over large areas and for long periods is the climate*."

Studies conducted in the Făgăraș Depression

In his 1945 paper, Pop presents research on the "diluvial" (Pleistocene) forests in Transylvania, respectively in the Avrig area - from Șipoțel and Valea Adâncata, from Săcădate, Sărata and Apoșdorf on the Rohrbach Valley (Tab. 1). In the author's opinion, the sequences from Adâncata reflect an interglacial forest evolution. Comparing the results to other European interglacials, the author states the possibility that the "interglacial" highlighted at Adâncata is Riss-Würm (Firbas, 1956), also called Eemian (Woldstedt, 1958), and that it is followed by the last glacial period, Würm (Penck & Brückner, 1901-1909). At Şipoţel, the configuration of the palynologically identified vegetation would suggest a cold and dry glaciation. The author also notices the lack of beech (*Fagus sylvatica* L.) in the layers from Șipoțel. There were no adequate conservation conditions in the Săcădate sequence and too little pollen was identified. At Sărata, the author considers that the deposit is allochthonous, representing the carbonaceous detritus brought by waters from nearby Pliocene layers. This assumption is based on the sporadic presence of pollen from *Carya* sp. Referring to the peat layers of the Rohrbach Valley, between Apoşdorf and Coveș, the author compares their age with that of the deposits in the Avrig area and concludes that they are part of the same interglacial. A cold, continental phase dominated by spruce is followed by a warmer one of beech (which, however, was not caught in the Avrig area). A new cold but wet phase follows, characterized by the presence of spruce and fir. The succession ends with the upward trend of pine, which indicates a climate that is more continental. Pop also presents various considerations on the framing of the results in the European alpine "glaciological" schemes. The author assumes a sufficiently low temperature in the last glaciation, which would have favoured the generalization of the pine forests and would have determined the descent to low altitudes of the subarctic-subalpine species. The author also considers that the tree line was lower than previously thought. Stronger glaciation could also lead to impoverishment in genera and species. However, maintaining the forest on the Romanian territory even during the last glaciation resulted in the rapid beginning of the first Holocene phase. It is characterized by the presence of local species that were maintained during the glaciation.

Table 1. Chronology of the studied sites

Palynological site	Location	The oldest attested climatic period								
		LGM	OD	LGI	YD	PB	B	AT	SB	SA
Adâncata	Făgăraș Depression	x								
Șipoțel	Făgăraș Depression	x								
Apoșdorf	Făgăraș Depression	x								
Dionisie Lake	Făgăraș Depression		x							
Blidul cu Poame	Făgăraș Depression									x
Avrig I	Făgăraș Depression		x							
Avrig II	Făgăraș Depression								x	
Porumbacu de Sus	Făgăraș Depression	x								
Mlaca Tătarilor Arpaș	Făgăraș Depression			x						
Bâlea I	Făgăraș Mountains									x
Bâlea II	Făgăraș Mountains						x			
Sărata	Făgăraș Mountains									x
Capra I	Făgăraș Mountains									x
Capra II	Făgăraș Mountains									x
Capra III	Făgăraș Mountains					x				
Capra IV	Făgăraș Mountains								x	
Podragu I	Făgăraș Mountains					x				
Podragu II	Făgăraș Mountains									x
Podragu III	Făgăraș Mountains									x
Podragu IV	Făgăraș Mountains								x	
Podragu V	Făgăraș Mountains									x
Puha I	Făgăraș Mountains									x
Puha II	Făgăraș Mountains									x
Oașa	Sebeș River Basin								x	
Prigoana I *	Sebeș River Basin					x				
Prigoana II *	Sebeș River Basin					x				
Mărgila	Sebeș River Basin								x	
Frumoasa Tărtărău	Sebeș River Basin							x		
Sălane	Sebeș River Basin							x		
Gura Sălanelor	Sebeș River Basin									x
Prigoana I **	Sebeș River Basin					x				
Prigoana II **	Sebeș River Basin							x		
Prigoana IV **	Sebeș River Basin							x		
Prigoana V **	Sebeș River Basin					x				

Abbreviations: LGM = Last Glacial Maximum; OD = Oldest Dryas; LGI = Late Glacial Interstadials; YD = Younger Dryas; PB = Preboreal; B = Boreal; AT = Atlantic; SB = Subboreal; SA = Subatlantic

Based on the results obtained in the study region and based on other studies conducted in Romania, the author emphasizes the role of the Romanian Carpathians in repopulating Central and Northern Europe with pine, spruce, birch, and other woody taxa.

In 1971 Pop published "The first radiocarbon dating in our Quaternary peat", in which he also refers to the Şipoţel deposit published in 1945 (Tab. 1). The sequence highlights two main stages. The older stage reveals the competition between pine and spruce; birch is present and with low percentages *Quercetum mixtum, Corylus, Alnus, Salix, Carpinus* and *Abies*; the more recent and more extensive stage is characterized by the domination of the pine (over 90%). In 1945 the author considered the sequence to be sedimented towards the end of the last Riss-Würm interglacial, evolving from a more temperate climate to the cold-continental climate of the Würm glaciation. The ^{14}C dating of the analyzed sample provided an age of 26,995 ± 360 yr B.P. Based on this ^{14}C date, the author reconsiders his previous assignment, from 1945, of this forest phase to the Riss-Würm interglacial. Consulting other datings of the European Pleistocene, the author concludes that the sample deposited during the Paudorf interstadial (± 27,000 years - Götzinger, 1935); this is a climatic period in which the forest elements listed above survived, a milder period than the previous one, and much warmer than the glacial stage that succeeded it. On this occasion, the author shows the need to determine the absolute age of all sequences and coal layers, both from Adâncata and Rora, but also from the ravines of other valleys in Transylvania.

Also, from the Avrig area, Bartmus publishes (1969a, 1995) the analysis of pollen from two eu-mesotrophic swamps, with very humid oligotrophic areas, called “Dionisie Lake” and “Blidul cu poamele”, located at an altitude of 440 m at the edge of Avrig (Tab. 1). The obtained sequences measure 7.20 m and 7.05 m in length, respectively. The sequence from "Blidul cu poamele" begins only in the Subatlantic, with the extension of the beech. The sequence "Dionisie Lake" (Avrig I) reveals the history of vegetation about 15,000 years ago. Basal pollen spectra were assigned to the end of the last glaciation. Bartmus identifies on this basis a *phase of the pine* (*Pinus* sp.) with the two sub-phases and the 6 episodes established by Pop in the Bilbor peat and in other regions (1932, 1936, 1943):

a. *The sub-phase of arid pine forests without thermophilic elements* comprises 4 episodes*:*

- the "Old dry pine forests" episode: 80-95.33% pine, accompanied by birch (maximum 14%) and willow (maximum 13.33%), spruce is constantly present, with small percentages;

- the "*Pinus-Picea*" episode: spruce reaches a maximum of 20%;

- the *Betula* episode: the rapid spread of birch, which records a maximum of 79.33%;
- the "New arid pine forests" episode: a new percentage increase of pine and the reduction of birch percentages; willow registers the maximum in the sequence, 19.33%;

b. *The less arid pine sub-phase* comprises *two episodes:*

- the "Pine forests with *Picea, Quercetum mixtum* and *Corylus*" episode: the appearance of more thermophilic elements (mixed oaks) and hazel (*Corylus avellana* L.); spruce reaches 24%;
- the "Pine forests with few spruce" episode: the increase of the percentages of oaks up to 20.67%, the curves of spruce and that of pine register a decrease.

After the *pine phase*, Bartmus highlights the *pine-spruce phase*, as a phase of transition from pine forests to mixed oak forests; pine registers a maximum of 54%, spruce 27%, and mixed oaks reach 47.33%, dominated by elm. In the *Picea-Corylus with Quercetum mixtum phase* Bartmus records two sub-phases and 4 episodes. In the first sub-phase mixed oak forests reach a peak, with the maximum of 53.33% (elm reaches 44.67%); fir, hornbeam and beech appear. The second sub-phase is characterized by the maximum of hazel, 180%. Within this sub-phase the author distinguishes 4 episodes:

- the episode *Quercetum mixtum-Picea-Pinus*;
- the episode with the domination of spruce over mixed oak forests;
- the return episode of oak, with *Tilia* predominance (19.33%), after the culmination of oak (13%);
- the episode of relative domination of spruce, with a maximum of 55.33%.

The hornbeam phase begins with the maximum development of hornbeam (65.33%); spruce persists by 20-30%, with a tendency to decrease continuously. The *beech phase* is less represented in this sequence of relatively low altitude; beech registers a maximum of 40%; its dynamics is complementary to that of the hornbeam to the surface; the fir tree (*Abies alba* Mill.) expands reaching values of 8.67% in the pollen spectra. In his work, Bartmus highlights several aspects. Except for hazel which has a maximum of 97.33% (possibly a local over-representation), beech is permanently dominant, competing with hornbeam, which exceeds it only on one level. The author also refers to the material processed by Pop but unpublished, collected in 1928 and 1943 from the swamp "Mlaca or Lacul Tătarilor" found in Arpașul de Sus. An analogy is attempted with the sequences from Adâncata, Șipoțel, Valea Rorei, Coveș -Apoșdorf (Agnita), as Bartmus elaborated a synthetic diagram.

At approx. 7 km from the sites near Avrig, close to the commune of Porumbacu de Sus, Boșcaiu *et al.* (1978) performs the palynological analysis of a layer of coal peat, interspersed between two alluvial layers of coarse gravel (Tab. 1). The base of the palynological diagram highlights two levels in which spruce dominates (maximum 80%), while pine and fir are poorly represented. The thermophilic elements did not exceed 2%. The authors consider these levels to belong to an episode with *Picea*, with a microthermal climate and a relatively high humidity; then the spruce curve decreases sharply, from 80% to 5%, complementary to the increase of the pine curve from 15% to 73%, which reflects a catastrophic evolution of the climate. These sequences accumulated in a dry episode of pine forests with little spruce and very little fir (0.7%); at its peak, the pine registers 87%. The attenuation of climatic rigor is indicated by an episode in which the pine decreases to 60% and the spruce increases to 37%; the thermophilic elements of *Quercetum mixtum* keep low values (1-5%). The last episode of the pollen diagram is that of the competition between pine and spruce; a more xeric sub-episode is distinguished, based on the amplitude of the oak and hazel curves, followed by a more humid sub-episode in which fir is affirmed (8%). In conclusion, the authors evoke the existence of a microthermal climate in Porumbacu, characterized by the domination of conifers and the significant lack of beech. The low values of the fir (8%) and of the thermophilic elements exclude the framing in the Eemian interglacial and the synchronization of this diagram with the one from Adâncata (Pop, 1945, 1971), where fir registers a maximum of 56%. The authors correlate this diagram with the base of the Șipoțel diagram (Pop, 1945, 1971) and consider that both sediments were formed during the Denekamp climate oscillation (Van Campo, 1969).

In 2006 Tanțău *et al.* publish the results obtained from the palynological analysis performed in the Avrig swamps (Tab. 1), also approached by Tanțău in 2003 and 2006 (doctoral thesis, respectively the published version of it). Two sequences of approximately 8.06 m and 11.90 m in length, from two adjacent peatlands near Avrig, were analyzed. Examining the obtained results, the paper concludes that the evolution of vegetation in the analyzed sequences, supported by 17 ^{14}C dates, begins in the Late Glacial period. This period includes the interval between Last Glacial Maximum (Walker, 1995; Litt *et al.,* 2003; Ravazzi, 2003) and Holocene. It is characterized on a regional scale by repeated climatic oscillations, which have led to significant changes in vegetation. The Avrig 1 sequence (A1) includes seven pollen zones assigned to the Late Glacial and seven Early Holocene pollen zones that have no equivalent in Avrig 2, a much younger sequence; also, in the A1 sequence, an oscillation was noticed that corresponds to a cold episode, dated for the first time in Romania (the "Avrig" episode); the A2 sequence begins only in the Subboreal. Based on the results obtained in these sequences, the authors present a detailed evolution of the vegetation in the region, making comparisons and references to the literature existing at that time in the country and abroad. In the Late Glacial period highlighted in the A1 sequence (Fig. 2), the authors reveal the existence of a steppe in the cold Dryas I period, characterized by the existence of *Pinus* and *Betula* populations scattered in the grassland vegetation. A ^{14}C dating of 13,880 ± 90 yr BP was obtained for this period. The authors use the name "Late Glacial Interstadial" for the period of moderate warming following Dryas I. The beginning of the climatic improvement of the Late Glacial Interstadial (probably Bølling), was characterized by an "open" arboreal vegetation, dominated by *Pinus*. A new cooling episode (Dryas II / Gerzensee oscillation) is characterized by steppe vegetation with small patches of *Pinus*. The obtained ^{14}C dating, too old in the authors' opinion to be attributed to Dryas II (^{14}C = 12,360 ± 70 yr BP) led to the authors naming this stage "Avrig". The vegetation highlighted for the last period of Late Glacial Interstadial (Allerød) consists of a “mosaic” of swamps and meadows, with specimens of *Pinus* and *Betula*. The presence of pine during this period has also been reported in other regions of Europe (de Beaulieu *et al.*, 1988; Willis, 1994; Goslar *et al.*, 1999; Nagy-Bodor *et al.*, 2000; Pokorny, 2002). The constant presence of *Picea* in the Avrig 1 sequence indicates the existence of this tree in glacial refugia in the region, as it was identified in other sequences in Romania (Fărcaș *et al.*, 1999; Björkman *et al.*, 2002; Tanțău, 2003; Tantau *et al.*, 2003), where its expansion began. The cooling characteristic of the last Dryas III stadial led to a strong decrease of *Pinus* and to the domination of the steppe grassland elements, similar to other Romanian sites. The ^{14}C dating obtained (10,300 ± 60 yr BP) confirms the assignment to this climatic period.

The restoration of the forest at the beginning of the Holocene took place gradually. Pine had the fastest response to climate change, followed by spruce and mixed forests were formed. Early elm expansion at the beginning of the Preboreal (^{14}C 9,670 ± 60 yr BP) was first recorded in low altitude areas. The expansion of the other elements of the mixed oak forests (ash, oak and linden) took place in the Avrig region after that of the elm, during the Boreal (^{14}C 8,550 ± 60 yr BP). The period of maximum expansion for hazel was recorded at the beginning of the Atlantic period (^{14}C 7,760 ± 50 yr BP), which, the authors say, would suggest its lack of glacial refugia in Romania. On the current territory of Romania, the hornbeam has its own phase, in the Subboreal as it expanded before beech. The regional differences between the history of the vegetation of Romania and that of Western Europe, e.g. the delay of the hazel phase, the hornbeam phase before the beech phase, etc., have already been noticed by Pop

(1942). The beginning of hornbeam expansion is dated to approx. 6,200 yr BP in the Avrig area. The first constant appearance of beech in Avrig suggests the presence of this taxon starting with 7,500 yr BP.

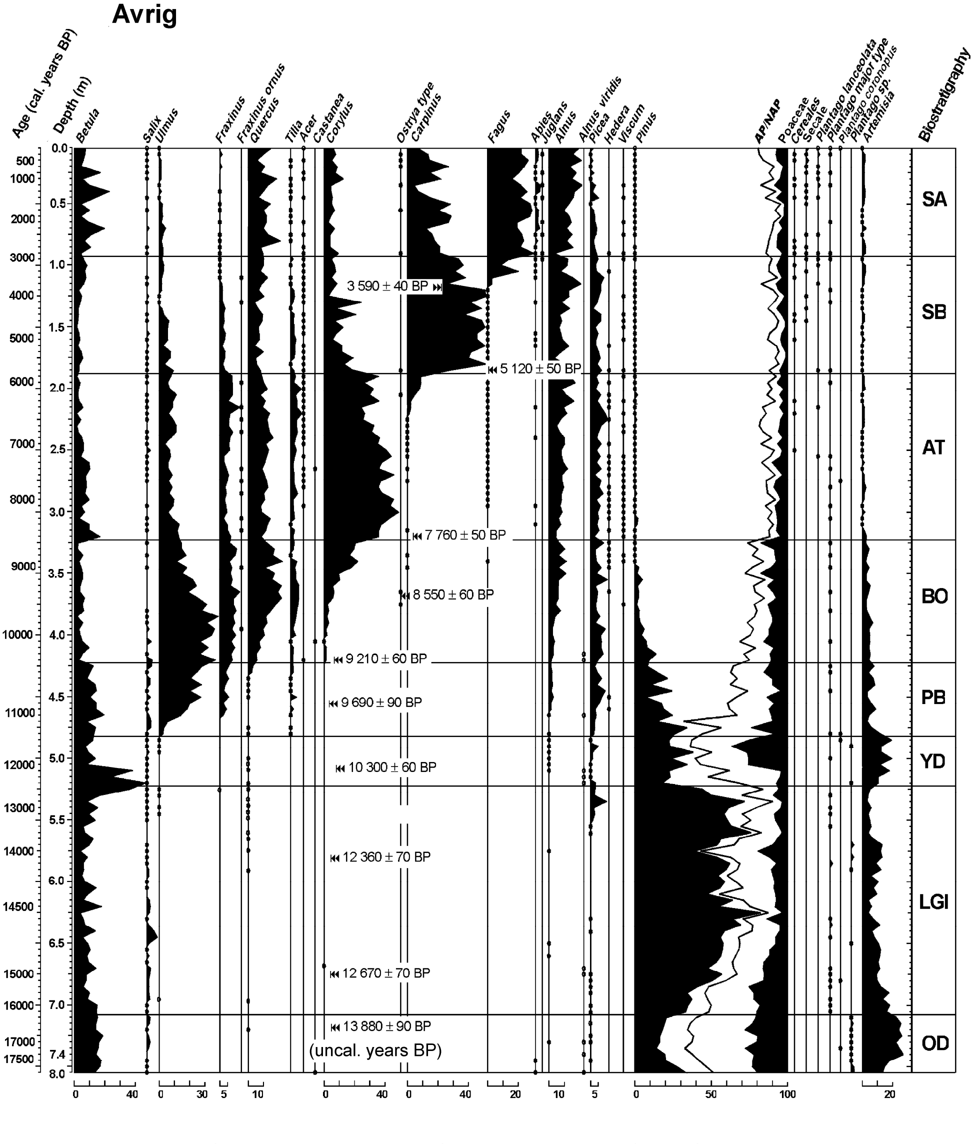

Figure 2. Simplified pollen diagram from Avrig, modified after Tanţău *et al.*, 2006: SA = Subatlantic, SB = Subboreal, AT = Atlantic, BO = Boreal, PB = Preboreal, YD = Younger Dryas, LGI = Late Glacial Interstadial, OD = Oldest Dryas

Its expansion took place much later, as in other locations in Romania. As in many other areas of Europe, human activity was one of the factors that facilitated its expansion and spread. The authors illustrate the difference between the appearance and expansion of a taxon in a region with the Avrig example, where beech survived individually or as small populations in the forest ecosystem for three millennia, before expanding into its own phase. Most palynological studies in Romania show a late appearance of the fir in forest structures, during the Subboreal period. This event was dated around 3,800 yr BP at Avrig. The first indications of human activity, respectively the first appearance of cereal pollen were observed in the A1 sequence, at about 6,000 yr BP.

Due to their geographical position, Romania and the Carpathian Mountains constitute a key territory from a biogeographical point of view, a contact area between Western Europe, its center and south, respectively the Balkan Peninsula. It has been claimed that the Carpathian territory was a favorable area for glacial refugia during the last glacial period (Huntley and Birks, 1983; Willis, 1994; Willis *et al.,* 1995; Diaconeasa and Fărcaş, 2002; Willis and Van Andel, 2004). During the Holocene, these areas were important sources for the recolonization of forests in Central Europe.

Tanțău *et al.* (2011) resume and detail a more recent sequence from Avrig. The history of vegetation begins in the Subboreal with the hornbeam optimum in the *hornbeam phase*, followed by the *beech phase* (Subatlantic) and is supported by four ^{14}C datings. The first indications of human activities in the region are visible in sequence with the first occurrence of cereal pollen, at about 4,200 yr BP. Some changes in the ecosystem have been caused by natural phenomena. Thus, a colder period that began about 600 years ago could be correlated with the LIA event (Little Ice Age, Mann *et al.,* 2009). However, the most significant changes have been associated, directly or indirectly, with human occupation. These effects induced by man included major deforestation, grazing, agriculture and man-made fires, whether voluntary or involuntary.

The peat bog from Arpașu de Sus has also been the subject of more recent studies, starting with 2004, when Fărcaș *et al.* publish the palynological analysis of a Subboreal age sequence. The “Lacul sau Mlaca Tătarilor” peat bog (540 m altitude) is located 3-4 km southeast of Arpaşul de Sus, Sibiu County, at the foot of the Făgăraş Mountains (Tab. 1). It was described by Pop (1960), who extracted samples from this bog, but did not publish the results. The flora is characteristic of mesotrophic bogs, with a pronounced oligotrophic character. A 16 m long sequence was extracted, and the authors (Fărcaș *et al.* 2004) present the palynological results obtained for the first 4 m from the surface. The analyzed sequence shows the existence of the last two forest phases, developed during the Subboreal and Subatlantic: *the hornbeam phase* and *the beech phase*. *The hornbeam phase* is characterized by the percentage increase of hornbeam, which during the Subboreal formed its own forest belt, between that of spruce and that of mixed oaks. The maximum percentage of hornbeam recorded is 58.98% reported to Σ A.P., respectively 53.54% reported to the total amount, ie Σ A.P. + Σ N.A.P. (sum of tree pollen - Arboreal Pollen grains - plus sum of grass pollen - Non-Arboreal Pollen grains). The increase of the hornbeam values is done to the detriment of the mixed oak. The values of the mixed oak reach 10.11% (respectively 8.74%). Beech registers a maximum of 29.74% (respectively 26.23%), and spruce is in turn well represented, with a maximum of 31.27% (respectively 28.95%), which exceeds that of beech. The authors note the first percentage occurrences of pollen from *Cerealia, Cannabis, Artemisia, Plantago* sp., which is evidence of human activity in the region, more or less distant. *The beech phase* in the Subatlantic is characterized by the percentage increase of beech and fir. Beech has developed favored by climate and human activity and has completed the stratification of vegetation in our mountains. The maximum percentage of beech recorded in its own phase is 58.36% (respectively 54.49%), exceeding the maximum value recorded by fir (12.80%, respectively 11.27%). However, this difference is also due to the low altitude of the analyzed site, respectively to the different distance from the vegetation belts of these trees. Beech remains at almost all levels at high values. There are two exceptions. In the first case, beech values drop to 19.30% (respectively 17.79%), simultaneously with the increase of the hornbeam percentage (45.06%, respectively 38.91%); this phenomenon is known in the Romanian palynological literature as the "subatlantic reaffirmation of hornbeam" (Diaconeasa and Fărcaş, 1998); in the second case, in the surface levels, beech values decrease to 11.79% (respectively 6.59%), simultaneously with the increase of those of spruce, birch, mixed oak, hazel and alder. This trend, associated with the sharp rise in grass pollen levels and the first occurrence of walnut (*Juglans*) suggestively reflects the anthropization of the region. The authors note the dominance of forest-type ecosystems in almost the entire sequence studied, examining the ratio Σ A.P./ total Σ (Σ A.P. + Σ N.A.P.).

Resuming the sequence from Arpașu de Sus partially published by Fărcaș *et al.* (2004), Tanțău *et al.* (2009) detailed other, older aspects of the history of vegetation in the region. The Late Glacial period has been characterized by several global and regional climatic oscillations. There are numerous studies, from Romania and abroad, that show a clear response of vegetation to Late Glacial climate fluctuations in Europe (Ammann and Lotter, 1989; de Beaulieu *et al.*, 1988; Feurdean *et al.*, 2007; Litt *et al.* ., 2003; Reille *et al.*, 1992 etc.). In Romania, as the authors point out, the succession of Late Glacial forests is known, largely due to the Palynological school in Cluj (Diaconeasa and Fărcaş, 1995-1996; Pop, 1929, 1932, 1942). The lack of absolute chronology (^{14}C dating) of these palynological sequences (except for Pop's 1971 work), is addressed and solved in modern palynological studies (Björkman *et al.*, 2002, 2003; Fărcaș *et al.*, 1999; Feurdean, 2004, 2005; Feurdean and Bennike, 2004; Tanţău, 2003; Tanţău *et al.*, 2006; Wohlfarth *et al.*, 2001). These studies have shown that there are differences between different sequences in different regions of the country, both between the time of appearance and the expansion of forest taxa. The analysis of the pollen from the base of the sequence from Arpașu, on a length of 4.30 m allowed the authors to reconstruct the history of vegetation starting from the Late Glacial period. Because the authors did not have ^{14}C dating of the sequence, the existing Avrig dating were used as a benchmark (Tanțău *et al.*, 2006). Dryas I, the first and oldest Late Glacial stadial, was not identified at Arpașu, but only in the Avrig sequence. The Late Glacial Interstadial includes milder climatic oscillations, the first of which is chronologically the Bølling oscillation, which is reflected in both sites. The Dryas II stadial was not observed in Arpașu. Allerød is the last interstadial phase of the Late Glacial, and was identified in both sites. Dryas III or Younger Dryas corresponds to an episode of climate deterioration and has also been identified in both sites (Avrig and Arpașu). For the base of the sequence from Arpașu, seven Local Pollen Assemblage Zones (LPAZ) were established, which were correlated with the eight LPAZ from Avrig, in order to punctually capture the changes of the vegetation in the region. Each pollen zonal boundary indicates significant changes in pollen deposition and represents major changes in vegetation cover. The beginning of the Bølling climate improvement was identified in both sequences, Arpașu and Avrig, being characterized by an open forest vegetation, dominated by pine, with the reduced participation of birch and willow. Isolated occurrences of spruce pollen resulted, according to the authors, from the transport due to air currents, from glacial refugia at lower altitudes. The steppe grasses (*Artemisia, Poaceae*) are declining. A climatic cooling episode, dated ^{14}C at Avrig in 12,360 ± 70 yr BP, is characterized, in both sites, by a steppe vegetation with *Artemisia, Poaceae* and *Apiaceae*. Pine, birch and willow have a dissipated presence. This oscillation is considered by the authors to be "too old" to correspond to the Dryas II period, hence the local name "Avrig". The vegetation of Allerød is a mosaic. The presence of pine and birch is explained by their high ability to colonize open areas in humid or dry environments. In Arpașu an intense development of the spruce forest, can be seen, which is delayed and more blurred in Avrig, probably due to the large distance between the site and the forest. Dryas III was registered in both Arpașu and Avrig. The forest vegetation is strongly affected, especially the presence of pine. The birch instead reaches a maximum that can be explained by local conditions: high humidity and lower temperatures. Spruce occurs sporadically in both sites, with frequencies not exceeding 1%.

In 2010 Tanțău *et al.* continue the publication of the results obtained at Arpașu, which palynologically reproduce the evolution of vegetation at the transition Younger Dryas (Dryas III) - Holocene, as well as in the early Holocene. As in the previous paper, chronozones refer to the absolute chronology obtained in Avrig and previously presented (Tanțău *et al.*, 2006). 9 Local Pollen Assemblage Zones were delimited. The first zones correspond to Dryas III, presented also in the previous sequence (Tanțău *et al.*, 2009). During Younger Dryas stadial there was an open forest vegetation, dominated by pine, accompanied by birch. At the beginning of the Holocene, in the Preboreal, the pollen diagram shows the decrease of the steppe grass communities (*Artemisia, Chenopodiaceae, Poaceae*), and a slight increase for *Pinus* and *Betula*. In the area, dense forests are gradually consolidating, forests in which pine, birch, and elm were the most common. Spruce, alder, oak and willow were also sporadically present in these early forests. In Arpaşu, as in other regions of the South-Eastern Carpathians, the low values of *Picea* pollen in the pollen diagram from the beginning of the Holocene are explained by the effect of the cold climate in Younger Dryas. The increase of the *Ulmus* pollen percentage in the beginning of the Holocene is similar to that found in other stations and suggests an early establishment of the elm tree in the Holocene. The early expansion of the elm tree may suggest, in the opinion of the authors, either the presence of small populations in the regional refugia, or the immigration from outside the Romanian territory. The expansion of other elements of mixed oak forests (*Fraxinus, Quercus* and *Tilia*) took place after the expansion of elm. Higher temperatures during the growing season played a significant role in their spread. In the Boreal, *Ulmus, Quercus, Tilia* and *Fraxinus* dominated the composition of the forests, and *Pinus, Betula, Alnus* and *Picea* were also present. At the end of the period, there is an increase in steppe grass communities, as well as a decrease in *Pinus* and *Betula* values.

The publication of results obtained in Arpașu (in the Mlaca Tătarilor bog) was resumed in 2020. Tanțău *et al.* present a palynological sequence, this time accompanied by ^{14}C dates. 5 Local Pollen Assemblage Zones were identified. The base of the sequence is not dated, but in the range 845-847 cm one ^{14}C dating was obtained: 9,110 ± 50 yr BP (9,515–10,087 cal yr BP), and towards the transition to the next area an age of 8,090 ± 40 yr BP (9,040 –9,466 cal yr BP); the vegetation is characteristic of the early Holocene, with a mixed forest dominated by *Ulmus* (20–40%); *Pinus, Picea* and numerous deciduous trees were present (*Fraxinus, Quercus, Tilia, Betula, Alnus*); Σ A.P. reaches 90% at the beginning of the sequence, then decreases to about 70%; the herbaceous elements are still poorly represented. Three ^{14}C datings accompany the next pollen zone, of which the last (5,125 ± 40 yr BP, respectively 5,789–5,937 cal yr BP) at the transition to the next zone; the percentages of A.P. increase; *Ulmus* remains dominant at first, then the percentages of *Corylus, Alnus* and *Picea* increase and *Ulmus, Betula* and *Tilia* decrease. *Pinus* and *Corylus* become dominant at approx. 8,500 cal yr BP; at approx. 7,200 cal yr BP *Carpinus* appears and expands. At the same time, the steppe herbaceous plants expand and diversify (*Poaceae, Apiaceae, Chenopodiaceae, Asteroideae, Artemisia*); there is a constant appearance of cultivated plants (*Cerealia*) and nitrophiles (*Rumex, Plantago lanceolata*). The following ^{14}C dating of 3,635 ± 35 yr BP (3,904–4,029 cal yr BP) attests to the presence of extensive mixed forests of *Carpinus* (30–50%) and *Picea* (20–40%); *Corylus* drops significantly, as well as herbaceous plants, below 15%. In the last local pollen zone, two ^{14}C datings were obtained, namely 3,480 ± 30 yr BP (3,679–3,802 cal yr BP), and further towards the surface 1,730 ± 30 yr BP (1,650–1,878 cal yr BP); in this interval there are extensive mixed forests of *Fagus* (40–60%) and *Carpinus* (20–40%); the percentages of *Picea* and *Corylus* decrease, *Abies* appears and expands and *Quercus, Ulmus* and *Betula* maintain constant percentages; the pollen zone towards the surface (45-0 cm) is accompanied by a ^{14}C dating at a depth of 34-36 cm: 645 ± 30 yr BP (387–549 yr BP); the percentages of *Fagus, Carpinus* and *Abies* decrease; the percentages of *Picea, Alnus, Corylus* and *Betula* increase; the percentages of herbs increase significantly (especially *Poaceae*), along with the anthropogenic indicators (*Plantago lanceolata, Cichorioideae, Asteroideae, Chenopodiaceae, Urticaceae, Rumex, Artemisia*) and the pollen of cultivated plants (*Cerealia* undifferentiated and *Secale*).

Studies conducted in the Făgăraș Mountains

In addition to the studies from the Făgăraș Depression, the studies carried out in the Făgăraș Mountains are also of interest, as the region is more difficult to access for such analyzes. We have selected in this paper some of the studies made in the Sibiu region and the immediate vicinity. They date back to the last century, but have not lost their validity. Although these studies do not have an absolute chronology, they serve as a starting point for possible follow-up studies, which would benefit from the support of modern, multidisciplinary methods.

Diaconeasa published in 1968 the results of the palynological analysis carried out in two peat bogs located in the Bâlea glacial cirque, on the place where former glacial lakes were located (lakes which have clogged in time), at approximately 2000 m altitude (Tab. 1). The palynological results are presented by the author in 4 complementary pollen diagrams. The first is an eutrophic swamp, of relatively recent age (Subatlantic), which tells the story of vegetation during the *spruce-beech-fir phase (Picea-Fagus-Abies)*. The second is an older mesotrophic bog (Boreal-Atlantic), larger and deeper, reflecting the peculiarities of vegetation during the last forest phases described by Pop: *the spruce phase with mixed oak and hazel (Picea-Quercetum mixtum-Corylus)*, *the spruce phase with hornbeam (Picea-Carpinus)* and *the spruce phase with beech and fir (Picea-Fagus-Abies)*. The author states that in high altitude peat bogs (1900-2000 m) the transition between the Boreal and the Atlantic is characterized by high humidity. This favored the expansion of spruce to higher altitudes, and determined a withdrawal of the elements of mixed oak forests towards the hill belt, respectively the narrowing of the *Pinus mugo* belt in the alpine zone. Spruce records in the pollen spectra of this transition stage register the maximum of the entire sequence (66%). Its pollen was transported by the ascending air currents from the spruce belt, having the upper limit at about 1800 m altitude. The elements of mixed oaks (*Quercus, Ulmus, Tilia*) remain at values of 16-20%. The author highlights the relatively high percentage of linden pollen, which reaches 8%, and which suggests in the respective levels an end of phase of the mixed oak. The sporadic appearance of beech and fir pollen, with subunit values, is explained by the author through a long-distance wind transport, while the appearance and increase of the value of *Carpinus* pollen attests to its existence at lower altitudes. *The spruce with hornbeam phase* corresponds to the Subboreal and is registered in a relatively thin layer, between the levels 95-80 cm, being characterized by the sudden decrease of spruce pollen from 66% to 50.66%, simultaneously with the increase of hornbeam pollen from 10 % to 22%. These fluctuations, concomitant with the decrease of mixed oaks pollen, suggest the role of hornbeam forests in changing the altitudinal limit of spruce and oak forests. Spruce is forced to climb to higher altitudes, and the oaks are forced to retreat to lower altitudes, in the hills and plains. During *the spruce with beech and fir phase (Picea-Fagus-Abies)* from the Subatlantic period, the author highlights 4 important moments in the pollen diagrams: the dominance of spruce pollen (64%) and the slight percentage superiority of fir pollen over beech; the same domination of spruce pollen, but with lower values (44%) and a descending trend (21%) simultaneously with the increase of beech pollen (31%); the dominance of beech pollen (56%) over spruce (13-21%) and fir (5-14%); the decline of beech pollen (27.33%) and the percentage return of spruce (40%). The author also finds differences compared to other high-altitude bogs in Retezat (Ciobanu, 1960; Pop *et al.*, 1966) and Parâng (Ciobanu *et al.*, 1967a, b). Thus, the pollen spectra from Bâlea lake show a high percentage of hazel, of 64%, during *the spruce phase with mixed oak and* *hazel*. Another peculiarity is the simultaneous appearance of beech and fir pollen in the second part of *the spruce hornbeam phase*, after which, during the last forest phase, beech begins to dominate in the pollen diagram (56%). The author also highlights the role of ascending air currents, which transported pollen from forests at lower altitudes to the alpine zone, where it was deposited on the surface of the bogs.

In 1969 Diaconeasa presents the results of the palynological analysis performed in the Sărata glacial cirque (Tab. 1). The described bog is oligotrophic, formed by the clogging of a former glacial lake, at an altitude of 1750 m, at the lower limit of the alpine zone. Peat began to deposit during the *spruce-beech-fir* phase, characteristic of the cold and wet Subatlantic. The author highlights the importance of this bog, as the pollen spectrum caught the maximum expansion of beech forests in the Făgăraş Mountains. The pollen diagram reflects the dynamics of the main forest taxa, with oscillating and complementary values, respectively spruce, beech and fir. At the level of 115 cm there is a pollen spectrum in which these three species have similar values (32% spruce, 29.7% beech, and 25.3% fir), while at a depth of 110 cm beech scores a first maximum, of 55.33%. Once installed, the beech forests show their supremacy over other forests in the region, although the bog is found approx. 100 m above the spruce forests, being closer to these forests than to the beech forests. The author states that the recent pollen rains of the forests from the alpine belt reflect fairly accurately the ratio between the surfaces occupied by the various belts of forest vegetation, within a radius of at least 20 km in a straight line. Thus, the antagonism between the percentages of spruce, beech and fir pollen reflects their dynamics in the peak period of beech forests. In the Sărata valley bog, the ratio between beech and spruce is sometimes 5/1, going up to 8/1 in the 45 cm level, where the beech pollen registers a maximum of 63.33%; this value was not found in previously published studies from bogs located in the alpine belt (Ciobanu *et al.*, 1967a, b, 1968; Diaconeasa, 1968). The author also explains the ratio between the presence of fir and beech pollen, a ratio in favor of beech throughout the sedimentation period; the higher weight of the large fir pollen disadvantages it compared to the other trees in the case of wind transport. The author states that the situations of slight subordination or equality of fir pollen percentage compared to spruce pollen percentage indicate a greater presence of fir compared to spruce in coniferous forests. The pollen diagram from Sărata also records the presence of pollen from dwarf mountain pine (*Pinus mugo* Turra, syn. *Pinus montana* Mill), mountain alder (*Alnus viridis* (Chaix.) DC), birch, oak, linden, elm, hazel, hornbeam and willow. As in other previously published bogs, the pollen of herbs (*Poaceae, Cyperaceae, Caryophyllaceae, Compositae, Umbelliferae, Ericaceae,* etc.) is present, known under the generic name of N.A.P., which indicates the succession of the meadows around the bog and of the herbaceous vegetation on the surface of the bog.

We also mention the studies carried out by Diaconeasa (1970) in the Capra glacial cirque, approx. 1 km away from Bâlea Lake, in the perimeter of 4 peat bogs, of different ages, located at approx. 2200 m altitude (Tab. 1). The analyzes performed revealed the same forest phases described above, with particularities that we will focus on below. It is important to note the older age of sedimentation in one of the bogs, which thus completes the picture of the evolution of vegetation in the Făgăraș Mountains. *The pine phase* is captured only in one of the swamps, called by the author the 3rd swamp, in a short layer of 15 cm, in a gray mud sediment. The allocation to this phase is made on the basis of substantial percentages of pine pollen, which reach a maximum of 86%. Together with spruce pollen (maximum 17%), conifer pollen in the levels assigned to this phase reaches values of 95-97%; the difference to 100% is represented by pollen of birch, willow, alder and mixed oak. The author attributes a Preboreal age to this stage, including it in the *sub-phase of less arid pine forests*, based on several arguments: the lack of hornbeam, beech and fir pollen; low percentages of grasses; the constant presence of pollen from mixed oak forests (oak, lime, elm and hazel species), evidence of global warming. The values of decreasing pine pollen (63%) and increasing spruce (28%) in the adjacent upperlevel, as well as the slight percentage increase of the mixed oak forests indicate the beginning of *the pine-spruce transition phase*; during this sequence, a sedimentation gap is recorded. The next chronological phase, the *phase of spruce with mixed oak and hazelnut*, is also not completely captured in the author's opinion, but probably only the end of it. It is characterized by relatively high values of hazel (16-24%) and oak (10%), with the addition of alder (8.66%), hornbeam, birch, fir and willow. The high percentage of spruce (70-78%), unlike the percentages registered in other bogs from the Făgăraș Mountains, is interpreted as a local expression of spruce expansion. The author does not exclude a possible under-representation of mixed oak forests pollen, with values influenced by the filtering effect of forests at higher altitudes. In the author's opinion, another cause for the under-representation of mixed oak forests, could be the mechanical processes of melting and draining of the snow on the surface of the bog, due to its slope, and the delayed deposition of spruce pollen compared to deciduous pollen. Another phase recorded in the pollen diagrams of the bogs studied in the Capra glacial cirque is *the spruce with hornbeam phase*, recorded complementary in these bogs. The maximum values recorded by hornbeam pollen are between 9% (bog 3 sequence a) and 20% (in bog 3 sequence b), but are even better represented in bog 4, where the phase of spruce with hornbeam is shown in more detail, on a thickness of 45 cm. Here the pollen of hornbeam has three maxima (23%), simultaneous with as many minima of spruce (45%), which, however, remain superior to hornbeam. The pollen percentages of fir and beech in this bog (15% and 8%, respectively) reflect their substantial spread towards the end of the phase. Mixed oak forests have cummulated pollen values of approx. 10%, of which oak (5-7%) and lime (3-5%) are better represented in the forests from lower altitudes. Pollen percentages of dwarf mountain pine and mountain alder (10%) indicate their rather modest presence in the glacial cirque. During the last chronological phase, *the spruce phase with beech and fir*, the author notes, as a feature of the Făgăraș Mountains, the simultaneous appearance of fir and beech in the pollen spectra, unlike the northern area of the Eastern Carpathians where beech appears before fir (Pop 1929) and unlike the southwest of the Southern Carpathians, where fir precedes beech (Ciobanu, 1948). The author emphasizes the continuous presence, with constant values, of beech and fir pollen which started in the previous phase of spruce with hornbeam, but also the competition between spruce, beech and fir during this last forest phase. At the beginning of this phase, the author highlights the "*initial episode of the spruce-beech-fir phase*", when these three elements have almost equal values in some levels: 32% spruce, 30% fir, 29% beech. In the pollen diagram Capra 5, fir pollen exceeds in some levels beech pollen, but not spruce pollen, levels framed by the author in the "*second episode of spruce-fir-beech*" of this phase. The surface levels captured the pollen of the neighboring forests during the peak of beech (49-52%), simultaneously with the decrease of spruce (20-33%) and fir (5-10%). This "*episode of beech-spruce-fir*" is followed by a fourth episode, recent and sub-recent, of "*spruce-beech-fir*", characterized by the reaffirmation of spruce (beech 35%, spruce 42-45%). The author shows in this paper that grass pollen (N.A.P.) fluctuates from one level to another and from one bog to another, but considers that the current alpine zone of the glacial cirque has never been occupied by spruce, and also, closer to present days, he considers that the expansion of open landscape has been noted. An interesting theory proposed by Diaconeasa in this paper is that the Făgăraș Mountains represent "the meeting point of fir and beech, as these trees immigrated to Romania on different pathways, the first from the Balkans, the second from the Atlantic region".

Diaconeasa continues the series of pollen-analytical investigations carried out in the Făgăraș Mountains within the doctoral thesis, and publishes in 1971 the results of the analyzes carried out in the peat bogs from the Podragu glacial cirque (Tab. 1). The palynological analysis of the 5 bogs shows that they began to form in the Holocene, but in totally different forest phases. Peat bog no. 1 began to sediment right at the end of *the pine phase*, but the sequence showed hiatuses. Bogs 2 and 3 date from the beginning of the Subatlantic, presenting a fragment from *the spruce-beech-fir phase*. Bog 4, very compact, began to sediment in *the spruce with hornbeam phase*. Bog 5 was deposited in the Subatlantic period, being relatively recent. The peat sediment at the base of bog I preserved 68% pine pollen, 20% spruce, 6% hazel, 4% mixed oak, willow, alder and birch. The author frames this base level at the boundary between Preboreal and Boreal, which corresponds, according to the author, with the end of *the pine phase*. In the nextlevel, characterized by the lower value of pine pollen (43%), and the increasing value of spruce (32%), mixed oak (15%) and hazel (15%), there is probably an incomplete *phase of pine-spruce transition*. Hornbeam, beech and fir pollen are missing. *The spruce with mixed oak and hazel phase* is well preserved in the following levels. In the pollen spectra, the pollen of spruce, mixed oak and hazel dominates. At the beginning of the phase, the elm pollen participates with 14-18% in the mixed oaks, a high value for an altitude bog; this value consolidates the results previously obtained by Pop (1929), Ciobanu (1948) and Diaconeasa (1969), and demonstrates that in the Făgăraș Mountains the elm tree initially had a dominant role among the mixed oaks. The percentage of elm pollen decreases as lime (9.33%) or oak (9.33%) pollen increase. This competition between elm and lime-oak suggests that the former became subordinate in the second part of the warm post-glacial (Atlantic) weather, when the values of mixed oaks decrease from 20-23% to 7.33%. The maximum percentage of spruce reaches 68.75%, while that of pine decreases to 29.33%. Hazel has the highest pollen percentage in the Atlantic (56.66%), a significant value compared to those recorded in the bogs of Parâng (Ciobanu *et al.,* 1967a) and Retezat (Pop, 1960) or even Semenic (Ciobanu, 1948) at lower altitudes. The author also reports the appearance of hornbeam pollen with 1.33%, which reaches 8% at the end of this forest phase, while fir and beech continue to be absent from the pollen spectra. *The phase of the spruce with hornbeam* corresponds climatically to the Subboreal and is recorded both in the peat of the first bog and in that of bog 4. The ascending curve of hornbeam pollen, from 8% to 17% in the pollen spectra of bog 1 reflects the final installation of hornbeam forests between oak and spruce forests. In the pollen diagram of bog 4 a maximum is registered for hornbeam (20.66%). Towards the end of the phase, beech and fir pollen appear in the pollen spectrum, with low values. *The phase of spruce with beech and fir* is represented in the pollen spectra of the 5 bogs analyzed. At the beginning of the phase, fir and beech occurred simultaneously, with modest values, an aspect highlighted in the pollen diagrams of the bogs 2-5; then there is an advance of fir, which records 23.33%, compared to beech's 15.33% (in bog 2). Spruce remains dominant, showing sudden oscillations at the beginning (44.66-55%). It is the *spruce-fir-beech episode* of this phase. The results obtained in the other swamps reflect the competition between spruce, fir and beech. *The beech-spruce-fir episode* is also highlighted, in which beech pollen (34%) exceeds that of spruce, while fir registers only 10%. The author considers that some studied bogs ceased their activity before beech dominated in this part of Făgăraș. Alpine grassland pollen was also recorded in palynological analyzes performed; alpine grassland associations were dominated by *Gramineae* and *Cyperaceae*, which are associated with species of the *Compositae, Caryophyllaceae, Umbelliferae* and *Ericaceae* families. The author states that in this glacial cirque and its surroundings the herbs are less represented in certain forest phases, and assumes that the forests covered larger areas compared to the alpine meadows.

The bogs of the Făgăraş Mountains also include those located in the Puha glacial cirque, on the border with Vâlcea county, published by Diaconeasa in 1972 (Tab. 1). In the Puha glacial cirque, the author found and explored two peat bogs. The first formed on a platform of glacial erosion at over 2200 m altitude and continues in the western part with a glacial lake. The second bog is 30 m below, and is fragmented by many streams. The author shows that both sediments began to deposit in the last postglacial climatic period (Subatlantic), in the *spruce-beech-fir* phase and that the results of the palynological analysis are slightly different from each other, due to the morphology of the terrain. Referring to the second bog, the pollen spectra of the base levels indicate a stage of domination of spruce (± 38%), which equals the percentage of fir and beech combined, while hornbeam is still well represented (maximum 14%). The upper level, at 90 cm, captures a moment when spruce and beech have equal values (26.66%), and mixed oak forests reach the maximum in the sequence, of 12%, while fir decreases to 6%. Towards the surface, beech pollen registers 3 successive and progressive maxima, up to 58%, contemporary with as many minima of spruce pollen, which decreases to 10%. The author considers these values as a clear indication that the peat sedimented during the maximum extension of beech. In this sequence, as in the sequence from Sărata (Diaconeasa 1969), fir is generally poorly reflected palynologically compared to beech, but participates with values almost equal to those of spruce (13.33% fir, 14% spruce). There are also exceptions to the rule, when the pollen curve of beech is disadvantaged (28%) by the more substantial assertion of spruce (26%) and fir (20%), for example in the level at 40 cm. Based on the pollen spectra obtained, the author finds that in most levels the pollen percentages of alder (*Alnus viridis*) are higher than dwarf mountain pine (*Pinus montana*); it is demonstrated that in the Puha glacial cirque the alder shrubs were much more extensive than the dwarf mountain pine shrubs, which the author observes in the palynological analyzes carried out in other alpine bogs from the Făgăraș Mountains. Another common denominator of these bogs is the decrease in the percentage of beech pollen towards the surface, concomitant with the revival of spruce pollen. The author also identified walnut pollen (*Juglans*) and edible chestnut (*Castanea*) in this bog, proof of the sub-recent existence of these trees in the surrounding intra-mountain depressions, with a milder and more constant microclimate. Comparing the palynological information obtained in the peat bogs from the Puha glacial cirque, with those in the Bâlea, Sărata, Capra and Podragu glacial cirques (Diaconeasa 1968, 1969, 1970 and 1971), the author states that they illustrate *the last episode of the spruce-beech-fir phase*, *the beech episode*.

Studies conducted in the upper basin of the Sebeș river

We further present two works, less known, published in the last century in the study region, this time in the upper basin of the river Sebeș. Bartmus publishes the palynological analysis of some bogs from the Sebeș Mountains (1969b), complementary to the analyzes carried out by Ciobanu in 1970 in the same region. The upper basin of the river Sebeș is one of the 10 regions of peat bogs established by Pop (1960) in the remarkable monograph on peat bogs in Romania, and these are, according to Pop, "the largest and most typical in the southern Carpathians." They are divided into three main groups: the first group with the bogs from the river meadows of the Frumoasa and Tărtărău valleys, tributaries of the Sebeș (1310-1320 m altitude), the second group with the bogs from the Sebeș and Sălanelor river meadows (1200-1210 m altitude), and the third group includes the highest bogs, located on the Prigoanei valley, a more northern tributary of the Sebeș (1360-1400 m altitude).

Bartmus analyzes in his work the bogs from Oașa, Prigoana and Iezerul Șurianului (Tab. 1). In the Oașa peat bog, located just above the confluence of Sălanelor and Frumoasa rivers, the author conducted 9 surveys, of which he analyzed three sequences. The Oașa III pollen diagram resulting from the deepest sequence of the bog shows even in the base levels a numerical superiority of spruce pollen (74-90%). The diagram reflects three sub-phases of forest evolution: *a spruce with hornbeam sub-phase*; *a sub-phase of spruce-beech-hornbeam and fir*; and *a spruce-beech sub-phase*. The first sub-phase, characterized by the domination of spruce, also shows a considerable participation of hornbeam, with a maximum of 18%. Beech appears only towards the end of this sub-phase. In the second sub-phase the curve of beech exceeds that of hornbeam, towards the end of the sub-phase. Fir has a significant evolution, registering a maximum of 14%. In the third sub-phase, spruce wins in front of the beech, registering even a remarkable maximum of

91%. The Oașa II diagram broadly reflects the same situation as the previous one, with the difference that pine is better represented in the basal spectrum (7.33%), and fir registers higher values (27.33%) in the 2nd sub-phase. The author concludes that the Oașa peat bog is relatively young, most of the peat being deposited in the Subatlantic. In the author's opinion, in these sequences the boundary between the two climatic periods was captured (the hot and dry Subboreal, respectively the colder and wetter Subatlantic), the so-called "grenzhorizont". In the Prigoana samples the author analyzed two palynological sequences, in peat bogs located on the left side of the stream (Tab. 1, Prigoana I*, Prigoana II*). At the base of the Prigoana I sequence, pine has a value of 52.67%, while in the Prigoana II peat bog, located upstream of the first, it is 91.33%, indicating a *pine phase*, followed in the levels immediately above by a *pine-spruce transition phase*. Following are the typical phases described by Pop, namely *the spruce phase with mixed oaks and hazel* (remarkable for the maximum of hazel, 70%), *the spruce with hornbeam phase* (29.3% maximum of hornbeam, maximum of fir at 14,7%) and *the spruce-beech-fir phase* (maximum 37% beech and 14% fir). With the exception of the basal levels in both sequences, in which pine dominates, the rest of the pollen diagrams are categorically dominated by spruce. In the same paper from 1969 Bartmus also refers to the results of the palynological analysis undertaken in the Mărgila peat bog, located at an altitude of approx. 1800 m, below Iezerul Șurianului; the author states that the base level was formed in the last period of *the spruce with hornbeam phase*. As arguments, the author invokes: the percentages recorded by the main tree species (spruce 71.33%, hornbeam 22%, hazel 10.67%), the dynamics of the spruce curve (ascending) and hornbeam curve (descending), with a last maximum of 32%. In the last forest phase, beech surpasses hornbeam, and in two levels it suprasses even spruce, while fir reaches the maximum in the sequence, 19.33%. The author states that beech could not reach domination in the last forest phase, as the peat bog was located at an altitude that goes far beyond today's upper limit of beech. However, the presence of beech pollen with high percentages in the pollen spectra shows that the beech forest was not far away.

In 1970, from the same region of the upper basin of the river Sebeș, Ciobanu presents the results of palynological analyzes performed in seven of the bogs described by Pop (1960): one from the first group (Frumoasa), two from the second group (Sălane and Gura Sălanelor) and four belonging to the third group (Prigoana I-II** and IV-V**) (Tab. 1). The Frumoasa valley harbored a series of bogs, located on the higher meadows of its banks, above 1300 m altitude. Ciobanu collected samples south of the canton of Tărtărău, on the right meadow of the valley, from the largest bog. The author states that the layers at the base of the sequence reflect an end of *the spruce with mixed oak and hazel phase*, respectively its last sub-phase, characterized by domination of spruce (69-78%), but also by the significant presence of mixed oaks (8 %) and hazel (10-12%). The pine curve starts with values of 10% and then gradually decreases, while alder reaches a maximum of 18%. The pollen of *Cyperaceae* and *Gramineae* is represented by the highest values in the sequence (23-30%, respectively 5.33-6.66%). *The spruce with hornbeam phase* was captured only in one level, with modest values for hornbeam (6.84%). Subsequent levels to the surface reflect a *spruce phase*, corresponding to the *beech phase* at lower altitudes. Throughout the sequence, spruce is dominant, reaching an maximum of 84%. The author notes the simultaneous appearance of fir and beech in the pollen diagram and the different evolution of the two taxa. The unbroken fir curve is characterized by very small values, while the beech curve, although much more modest than in other sequences at similar altitudes in the southern Carpathians, reaches values between 4 and 20%. On both their banks, the Sălanele (tributaries of the Sebeș on the left side) harbour a large number of peat bogs, of which Ciobanu extracted samples and analyzed two sequences, "Mălștina Sălane", and the peat bog called by Ciobanu "Gura Sălanelor" located near the outflow of Sălan into the Sebeș river. The diagram from Sălane reflects at its base the end of *the spruce with hazel phase*, and in the immediately next level a snapshot from *the spruce with hornbeam phase*. Hornbeam reaches the value of 14.66%. In the same phase, beech and fir simultaneously appear, and together with hornbeam they cause a sudden decrease in spruce values, from 95% to 62.66%. The rest of the pollen diagram reflects the last phase from the evolution of forests in the region, which Ciobanu calls *the new spruce phase*, and which corresponds, at lower altitudes, to *the beech phase* (from the Subatlantic). The reflected *episode* is one of *spruce*-beech, characterized by low values of fir (2-10%), compared to those of beech (11-42%); they, at some point, surpass those of spruce (42% compared to 36%). At Gura Sălanelor the peat deposit is much younger, belonging to the last forest phase. The first levels reflect a spruce forest (93-98%), accompanied by pine, fir, beech, hornbeam, all with low values, below 3.33%. Starting from 145 cm to the surface, the diagram reflects *the spruce-beech-fir sub-phase*, with a last *episode of spruce-beech*. During this sub-phase, spruce always remains dominant, with values ranging between 56 and 98%. Fir and beech, present in the beginning with modest values, begin to assert themselves progressively. The beech curve has an upward trend, culminating in a maximum of 27.33%. Fir, which appears simultaneously with beech, spreads faster than beech, but at some point its curve is exceeded by that of beech and remains modest (maximum 15.33%). Like Bartmus (1969b), Ciobanu (1970) analyzes the succession of vegetation in the bogs from the

Prigoana meadow (Prigoana I-II and IV-V). Prigoana I and V bogs are located on the right meadow of Prigoana, while Prigoana II and IV are located on the left meadow. The Prigoana I peat bog, located high on the ridge, reflects older aspects of the history of forests in the region, namely three distinct phases, *the pine phase*, *the spruce with mixed oak and hazel phase* and the more recent *phase of beech and fir*. *The pine phase* is characterized by very high values of pine (80%), along with spruce (21.33%), birch, alder, willow and hazel. The elements of mixed oak forests are missing. The author considers that this is the "*less arid sub-phase of Preboreal pine forests*", respectively "*the episode of pine forests with few spruces*" (Pop 1943). *The pine-spruce transition phase* is recorded at a single level, and is characterized by a rapid increase in spruce pollen values (at 53.33%) simultaneously with a sudden decrease in pine (at 36%), but also by the significant increase of birch and hazel and the appearance of mixed oak forests. *The spruce with mixed oak and hazel phase* is characterized by spruce domination, which reaches a maximum of 86%. The relatively low values of mixed oaks (maximum 10%) and hazel (maximum 25%) indicate *the last sub-phase*, namely the "*absolute maximum of spruce, with a moderate spread of mixed oaks and hazel*" (Pop 1942). The author emphasizes that the order of occurrence of the elements of mixed oaks is elm, linden, oak, an aspect reflected in several pollen analyzes from Romania. Hornbeam, with an uninterrupted curve but with low values, reaches a maximum of only 8.66%, so it is considered that *the spruce with hornbeam phase* could not be captured in this sequence. *The recent spruce phase*, represented by the *spruce-beech-fir sub-phase*, reaches the surface of the sequence and shows the dominance of spruce in the forests of the region (minimum 50%), but also its dynamics in competition with beech and fir. Fir is represented by quite low values, which reach 12.66%, while beech reaches two highs of 28.66% and 32%. In general, its curve is antagonistic to that of fir, but both undermine the percentage representation of spruce in the diagram. About 2 km south of the first peat bog, Prigoana I, Ciobanu collects and analyzes samples from the bog he calls Prigoana V, finding a great similarity between their pollen diagrams. In Prigoana V, however, it is worth noting that in *the pine phase*, there is a higher percentage of pine in the basal level (90%) and a lower percentage of spruce (9.33%) that can be noticed, which attests to a slightly older age of this sequence; in *the spruce with mixed oak and hazel phase*, spruce registers its maximum in the sequence, of 91.33%; elm and hazelnut culminate simultaneously (12.66% and 36.66% respectively) causing a sudden decrease in spruce values (from 88% to 61%); birch also contributes to this decrease, reaching at the same level a maximum of 18.66%, unique in the region; in *the spruce-beech-fir phase* the continuous curves of fir and beech begin simultaneously (with maximum values of 12.66% and 24.66%, respectively). In the analyzed bogs located to the left of the Prigoana valley (Prigoana II and IV) the pollen diagrams reflect in the basal levels *the end of the spruce with mixed oaks and hazel phase*. The high values of thermophilic essences are noticeable, oak reaching 18%, the highest percentage of all the seven diagrams, and hazel 32%, a maximum surpassed only in the Prigoana V sequence (36.66%). *The spruce with hornbeam phase* follows, the author noting that in Prigoana II sequence it is the best represented of all the seven diagrams analyzed. Hornbeam reaches a maximum of 22% in this phase. In the last forest phase, the sub-phase reflected in both diagrams is that of *spruce-beech-fir*, much better outlined than in the other bogs analyzed here. Fir has the highest values in the region (15.33% for Prigoana II, 22.66% for Prigoana IV); beech also reaches the highest values in the region (37.33% and 32%, respectively), having as a complementary effect in the pollen diagrams the significant decrease of spruce values (below 50% on average). The values of the identified herbaceous pollen are low, reaching a maximum of 30%, which shows the predominance of the forest in each level analyzed.

Conclusions

The oldest vegetation records from the Upper Pleistocene in the Sibiu region are those from the Adâncata Valley and from Şipoţel, those from Săcădate, and from the Rohrbach Valley in Apoșdorf. They probably reflect images of the vegetation from the Late Glacial Maximum and are characterized by the dominance of forests in the interstadial periods and by the open landscape of forested steppe in the cold, stadial periods. The oldest episode at Adâncata shows a spruce and fir forest, with a lot of oak and lime, with alder, pine, birch, hazel and few willow trees. The analyzes highlight the competition between spruce and fir. The sequence from Șipoțel shows two main stages: an older stage of competition between pine and spruce, when the forest also included birch and disseminations of mixed oaks, hazel, alder, willow, hornbeam and fir; the more recent and more extensive stage (extensive as duration) highlights the dominance of pine. In Apoșdorf, a cold and continental phase dominated by spruce is followed by a warmer one, with beech, which was not represented in the Adâncata and Șipoțel sequences. A new cold but wet phase follows, characterized by the presence of spruce and fir. The succession ends with the upward trend of the pine, which indicates a more continental climate. In the layers of coal peat discovered near Porumbacu de Sus, the existence of a microthermal climate is revealed, characterized by the domination of conifers. This episode of vegetation evolution can be correlated with the basis of the diagram from Șipoțel. The total lack of beech is also significant. In the Late Glacial, the succession of stadial vegetation (Dryas I-III) was registered, respectively the succesion of interstadial vegetation (Bølling-Allerød complex). The succesion was more or less incompletely captured in the sequences from Avrig and Arpașul de Sus, supported by ^{14}C dates. During the stadials, in the study region an open, steppe vegetation is revealed, characterized by the existence of isolated populations of pine and birch, scattered throughout the herbaceous vegetation. During the interstadials, there is an open forest vegetation, dominated by pine, with a low participation of birch, willow and spruce. The history of the Quaternary vegetation from the Holocene in the Sibiu region and the surrounding areas fits broadly in the classical succession known for Romania, for the Carpathians and the surrounding areas of medium and low altitudes: the end of the pine phase, respectively a pine-spruce transition phase in the Preboreal period; the spruce with hazel and mixed oaks phase in the Boreal-Atlantic period; the spruce-hornbeam phase in the Subboreal; the spruce-beech-fir phase in the Subatlantic. Supporting the history of vegetation in the Sibiu region with an absolute chronology was possible since modern palynological studies. The differences and similarities between the percentages of taxa were highlighted, but also between the moment of appearance and expansion of different plant taxa in the different sites studied. Also, the determining factors of the vegetation evolution were analyzed, both natural, especially climatic but also edaphic, geographical, micro-morphological, etc., as well as those related to human intervention.

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=== SHORT COMMUNICATION ===

What is for dinner? Qualitative and quantitative data regarding small mammal species identified in pellets of long-eared owl (*Asio otus*) from Cluj-Napoca

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Abstract. Analysis of pellets provides information on the prey distribution, abundance, behavior and trophic relationships between Strigiformes and small mammals. By analyzing the skulls from long-eared owl (*Asio otus*) pellets, collected from a colony located in the Mercur Park, in Cluj- Napoca, Romania, we aimed to identify the small mammal species and to compare the results with available ones from another study conducted 2 years ago in the same area. During 7 collecting campaigns (November 2018 to March 2019) in 538 pellets a total of 1290 skulls were identified. Over the study period, the colony increased from 10 to 40 birds. Six species of small mammals were identified: the field striped mouse (*Apodemus agrarius*), the yellow-necked mouse (*Apodemus flavicollis*), the wood mouse (*Apodemus sylvaticus*), the European water vole (*Arvicola terrestris*), the field vole (*Microtus agrestis*) and the common vole (*Microtus arvalis*). Compared with the previously study, a new species was identified: the yellow-necked mouse and another one was missing: the bank vole (*Myodes glareolus*).

Keywords: Strigiformes, diet, rodents, anthropogenic impact

Introduction

Pellet analysis is a valid method used in the field of owl ecology to obtain data on the small mammals' species. The pellets provide information of the prey distribution, abundance or behavior. Moreover, the trophic relationships between Strigiformes and small mammals can be assessed, as the diet composition of these birds have geographical and climatic variations (Selçuk *et al.,* 2017).

Long-eared owl (*Asio otus* Linnaeus, 1758) is spread throughout Europe, North-West Africa, Asia and North America. In Romania, long-eared owl is one of the most wide-spread strigiform species (Marks *et al.,* 1994; Halici and Stermin, 2020). The populations from the northern parts of its range are migratory and those from the southern and western are sedentary (Cramp and Simmons, 1985). Typical of the long-eared owl is that they form large wintering colonies, in urban and suburban areas, where they can find a stable source of food, protecting themselves more easily against weather conditions and potential predators (Cramp and Simmons, 1985; Galli *et al.,* 2015; Lövy and Riegert, 2013, Moga *et al.,* 2005; Halici and Stermin, 2020).

Long-eared owl diet is made up of 90% of voles, but they can eat sparrows or other birds when the preferential small mammals are low in numbers (Iozon *et al.,* 2002).

Undigested food is relapsed in the form of pellets that contain mostly rodent residues. The pellets have round margins as well as a round surface. Their dimensions can reach 4.8 cm in length and 2.5 cm in thickness at most. Pellets are found in large numbers during winter when the owls gather in groups (Olsen, 2019).

Comparative analysis of the data obtained on long term studies can provide new insights into the structure of animal communities and the changes that occur in population features in relation to bioclimate (Tores and Yom-Tov, 2003). Therefore, understanding the diet of these birds, as well as the dynamics of the predated species are key elements in conservation efforts (Iozon *et al.,* 2002; Birrer, 2009).

In this context, the aims of our study were: (i) to evaluate the number of pellets and the skulls collected from the colony over the wintering periods (ii) to identify the small mammal species by skull analysis and (iii) to compare the results with data collected from another study conducted 2 years ago in the same area (Margea, 2018).

Study area

The colony was located in the Mercur Park, from Gheorgheni district in Cluj-Napoca on Detunata alley at 46° 76' N, 23° 62' E, behind the Mercur Complex, but also next to it at 46° 46' N and 23° 37' E. This whole area is considered „an urban forest", frequented daily by the elderly who walk their pets. The identification of the colonies was performed with the help of the locals who are familiar with the presence of owls in the area as the colony gathers there every year.

The vegetation in that area is dominated by pines (*Pinus*). This is one of the long-eared owls preferred species, thus becoming a place frequented annually by them.

Materials and methods

Field data were collected from November 2018 to March 2019. At each campain the long-eared owls located on the study area were counted and all the pellets collected. Observations were made during the day light to increase the possibility of locating birds in the resting site.

The collected pellets were dried in paper-covered trays. The pellets were measured in length and width using a ruler and a bow compass.

Each bone from the pellets was separated and cleaned with tweezers. The skulls and the mandibles found were also separated according to the date and location for further examination.

The skeleton parts from samples were analyzed using a binocular magnifier (Optika) and a rodent determinator, based on the dentition, using the book Mammals of Poland (Pucek, 1981).

The distinction between the species: the field striped mouse (*Apodemus agrarius*), the yellow-necked mouse (*Apodemus flavicollis*), the wood mouse (*Apodemus sylvaticus*) was based on the differences in their dentition according to Pucek, 1981.

Results and Discussions

A total of seven collecting campaigns were carried out in the Mercur Park area from November 2018 to March 2019. Over the study period, the colony increased from 10 to 40 individuals.

During the five months, a total number of 538 pellets were collected. The maximum number of pellets (n= 222) was recorded in January 2019, with a strong increase from November (n = 9) followed by a slow decline in February (n = 180) and March 2019 (n = 127) (Fig. 1). The small number of pellets collected in December can be explained by the fact that the samples were collected in early December (09 December 2019) when the birds had not yet settled in the area.

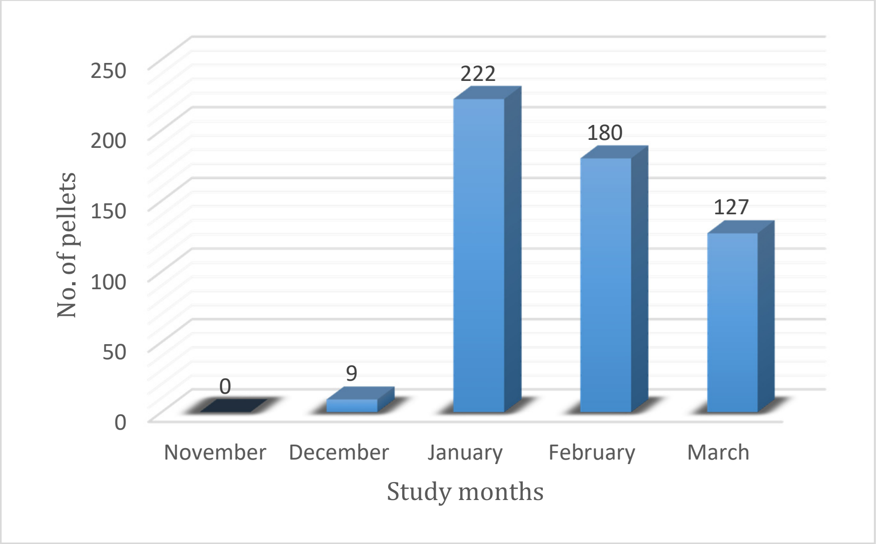

Figure 1. The monthly dynamics of the number of colected pellets.

A total of 1290 skulls were identified in the pellets during our study, with a minimum of 14 (December) and a maximum of 576 (February) (Tab. 1, Fig. 2).

Table 1. The minimum, maximum and standard deviation (S.D.) of the number of collected pellets and skulls during the months when the samples were identified on the field (December, January, February, March)

	Pellets	**Skulls**
min.	9	14
max.	222	576
med.	115.5	295
S.D.	100.01	247.29

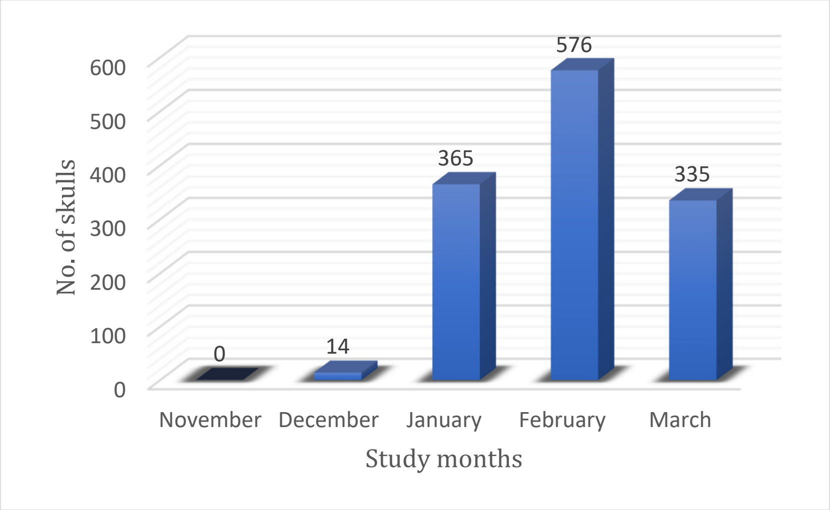

Figure 2. The monthly dynamics of the number of skulls

Six species of small mammals were identified: the field striped mouse (*Apodemus agrarius*), the yellow-necked mouse (*Apodemus flavicollis*), the wood mouse (*Apodemus sylvaticus*), the European water vole (*Arvicola terrestris*), the field vole (*Microtus agrestis*) and the common vole (*Microtus arvalis*). The common vole (82.96%) was predominant, followed by the yellow-necked mouse (10.32%) (Tab. 2). The previously recorded data (Margea, 2018) showed similar number of taxa but with differences in species: the field striped mouse, the wood mouse, the European water vole, the bank vole (*Myodes glareolus*), the field vole and the common vole, mainly the common vole and the field vole.

Table 2. The number of individuals from each species identified in the months when the samples were identified on the field

	December	January	February	March
Apodemus agrarius	0	21	20	2
Apodemus flavicollis	4	40	45	17
Apodemus sylvaticus	0	4	6	0
Arvicola terrestris	0	0	6	1
Microtus agrestis	0	12	3	1
Microtus arvalis	5	221	437	195

Comparing our results with the previously one, in our study a new species was identified: the yellow-necked mouse and another one was missing: the bank vole (Tab. 3).

Table 3. The precence (+) and absence (-) of small mamal species determined in the two studies

Species	2016 – 2017 (Margea, 2018)	2019 - 2020
Apodemus agrarius	+	+
Apodemus flavicollis	-	+
Apodemus sylvaticus	+	+
Arvicola terrestris	+	+
Microtus agrestis	+	+
Microtus arvalis	+	+
Clethrionomys glareolus	+	-

Analyzing the number of species related to the time periods, in November, there were no pellets recorded and after the birds began to gather in the colony, the low number of small mammal species was identified in December (n = 2) and the maximum number in February and March (n = 6) (Tab. 2).

The most predominant species was the common vole (n = 858) followed by the yellow-necked mouse (n = 106) and the least common was the European water vole (n = 7). The minimum number of individuals was found in December and March, indicating the periods when the owls returned to, respectivly left Cluj-Napoca.

With a percentage of about 82.96%, the predominant species in the diet of long eared-owls is the common vole, similar with the previous study – 91.19% (Margea, 2018). Another numerous species between November 2018 and March 2019 was the yellow-necked mouse (10.32%), unlike the previous study (Margea, 2018) where during the winter of 2016 - 2017 the field vole (6.22%) was the second species encountered. The species with the lowest percentages are represented by the wood mouse (0.97%) and the European water vole (0.10%) in the current study while in the previous one, the field striped mouse (0.26%), the European water vole (0%) and the bank vole (0.52%).

A major difference between the two studies is the presence of the bank vole in the previous one. The bank vole prefers forest habitats and coastal areas, being a rare presence near the cities (Benedek, 2014). A possible explanation for their observation near the city is the increase in the density of the area or the need for food in the conditions of a harsh winter. Also, the bank vole may be present in the pellets in 2017 and absent in 2020 because of a sampling error given the low number of pellets collected. To observe if it is a real change in the small mammal community further research is needed.

Following the analysis of the pellets, three skulls of shrews and a beak of a common chaffinch (*Fringilla coelebs*) were discovered, along with some remains of plastic bags and cigarette filters. Birds are an alternative source of food for the long-eared owls and the remains of materials (plastic and cigarette filters) indficate that the urban environment in which the long-eared owls settled shows signs of pollution.

The factors that influence the presence or absence of some species in the diet of long-eared owls be a long and harsh winter and the anthropogenic impact on the wintering habitats. Moreover, climate influences the availability of food. To survive, long eared-owls adapt by changing their diet or extending their hunt ground. The plastic found in the pellets present an alarm sign as the polluting behaviour of humans affect the quality of food ingested by the long-eared owl.

Conclusions

The qualitative analysis of pellets, revelead that the diet of long-eared owl from the colony in Cluj-Napoca was composed of six species: the field striped mouse, the yellow-necked mouse, the wood mouse, the European water vole, the field vole and the common vole. The yellow-neck mouse is a species in addition to the previous study.

The diversity of the owl's diet is indicated by the presence of shrews skulls and the beak of a common chaffinch. Also, the impact of human activity is observed through the plastic remains found in the pellets.

Taking into account our results and the previous study (Margea, 2018), the presence or absence of some species in the diet of long-eared owls present in the Mercury Park area depends on a variety of factors. Those factors could be the climate - a long and harsh winter and the anthropogenic impact on the wintering habitats.

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